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MODERN CORN BREEDING AND PRODUCTION IN MEXICO[1]

E. J. Wellhausen[2]

Mexico is a mountainous country with many different climates. Its size is about ¼ that of the United States, but only about 7.6 per cent of its total area, or 37,316,000 acres, is tillable. The remainder is either too dry or too mountainous.

From an agricultural viewpoint the country comprises a number of well-defined natural regions. The Northern Mesa and the Central Mesa constitute what is known as the great plateau of Mexico. This varies in elevation from about 4,000 ft. in the north to an average of about 8,000 ft. in the south central region, and comprises about ¾ of the total area of Mexico. The great plateau is bordered on the east, west, and south by mountainous escarpment areas with elevations up to 18,000 ft. The southern and Chiapas highlands are also very rough and mountainous but somewhat lower in elevation. Along the Gulf is a long, comparatively narrow strip of lowland called the Gulf Coastal plains. On the west coast the mountains virtually rise out of the sea, except in the north where there is a wider strip of low level land called the Sonora desert and plain. Lower California is much like the Sonora desert.

The Northern Mesa is a continuation of the basin and plateau country of Arizona, New Mexico, and western Texas. It consists chiefly of vast stretches of nearly level land covered with mesquite and cactus. Its low rainfall of 5 to 20 in. with a very irregular distribution makes it of little value for agricultural purposes, except for a few small irrigated regions devoted to wheat and cotton production. The Northern Mesa comprises about ½ the total area of Mexico but contains less than 1/5 the population.

In sharp contrast is the region to the south known as the Central Mesa, where over half of the people live in an area comprising about 15 per cent of total Mexico. The difference is rainfall; in the Central Mesa the average rainfall between June and October varies from 20 to 40 in., which together with its 7 or 8 extensive flat-bottomed valleys has made this area the granary of the nation.

Although corn is grown throughout Mexico wherever people live, about 70 per cent of the commercial corn is produced in a belt across Central Mexico from 18° to 22° latitude. This area varies in elevation from 0 to 10,000 ft. and in rainfall from 5 to 120 in. These conditions have been conducive to the development of many different races and varieties of corn. The average yield is from 9 to 12 bu. per acre—not enough to feed the Mexican people.

About 85 to 90 per cent of the total corn produced in Mexico is used directly for human consumption. It is soaked in limewater overnight, washed, ground wet, and fashioned into pancake-like patties called tortillas. Tortillas, together with beans and chili peppers, constitute the main diet of 65 to 70 per cent of the 25,000,000 people. As much as a pound of tortillas per person may be eaten at each meal when sufficient corn is at hand. When taken in such quantities, tortillas provide a fair amount of nourishment, but present corn production does not often permit consumption at this rate.

The low average yield of corn in Mexico is largely due to 4 factors.

1) The first and most important is depleted soil fertility. Through continuous mono-cultivation of corn on the same piece of ground, the mineral nutrients, especially nitrogen and phosphorus, have become practically exhausted on much of the cultivated land. In many areas yields have leveled off at a point determined by the nitrogen that falls from the air with the rains, or fixed by certain leguminous weeds that might be permitted to grow between the rows. The response after the addition of fertilizer is often tremendous. In certain areas the application of nitrogen and phosphorus makes the difference between a good crop and no crop at all. In general, yields throughout Mexico can be greatly increased solely by the use of chemical fertilizers.

2) A second important factor in low average corn yields is that most varieties have a low yield potential. Most of the native varieties of corn now existing in Mexico are low in yield capacity and have become adapted to low fertility conditions through many years of natural and artificial selection. For many centuries this process has been operating for those characters which would allow a variety to produce something under extreme conditions of drought, and low soil fertility. Over the years low yields have meant hunger, but a complete crop failure has meant starvation. Since it has been impossible to store corn from 1 season to the next because of excessive weevil damage, a bumper crop in any 1 year in ancient times did not have the appeal that it does today as a commercial commodity that can be converted into money. The tendency has been through natural and artificial selection to develop varieties that were sure to yield something from year to year, rather than a great deal one year, little the next, and even nothing when conditions were extreme.

[1] Invitational paper presented September 9, 1953, at the Symposium on Cooperative Agricultural Research in the Western Hemisphere, arranged jointly by The American Phytopathological Society, The American Institute of Biological Sciences, and The Potato Association of America, at the 45th Annual Meeting of The American Phytopathological Society in Madison, Wisconsin.

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[2] Local Director of the Mexican Agricultural Program of The Rockefeller Foundation.

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TABLE 1.—*Comparative Yields of Native and Improved Varieties of Corn With and Without Fertilizer on Depleted Soils*

	Yield (unfertilized)		Yield (fertilized)	
	Native Variety	Improved Variety	Native Variety	Improved Variety
Kgs. per hectare	1,000	1,250	3,500	7,000
Bu. per acre	16	20	55	112

3) A third factor perhaps less important than soil and varieties is primitive cultural methods. The majority of the farms are about 10 acres in size. On these 10 acres each family annually produces corn, interplanted with beans, in an attempt to supply the yearly food requirements. The head of the family has no money for machinery. A few of the more fortunate families may have steel plows and a pair of mules, but by far the majority depend upon the ancient Egyptian plow. This plow is homemade and used for all operations—plowing, planting, and cultivating.

4) A fourth factor contributing to the low average yield of corn in Mexico is the lack of moisture. The amount of rainfall is variable from year to year and often poorly distributed. Much of the water that falls runs off because of the poor physical condition of the soil, brought about through continuous corn culture. Not much can be done to increase the rainfall, but twice as much corn as is now grown in Mexico can be produced under present rainfall conditions through the improvement of soil fertility and through the development of higher yielding varieties adapted to the higher levels of fertility. At the same time as higher yielding varieties are developed, the soil will need to be improved, or vice versa. Improvement in either factor alone will be only partially efficient, as shown in Table 1.

On unfertilized soil an improved variety will yield little more or often not as much as a native variety adapted to the low fertility conditions. On fertilized or otherwise improved soil the same native variety may yield 2 or 3 times as much, but its maximum yield capacity is soon reached at a point much below that of an improved variety bred for higher fertility. Many of the improved varieties or hybrids yield up to twice as much as the native varieties at improved levels of soil fertility.

Varietal improvement for each of the principal corn-producing regions of Central Mexico was started more or less simultaneously in 1944 as a cooperative project of the Ministry of Agriculture and The Rockefeller Foundation. Breeding stations were established in zones at 1,500-ft. intervals of elevation from sea level to 8,500 ft. The objective has been gradual improvement which could be obtained in steps and passed on to farmers immediately.

The first obvious step in each elevation zone was the collection and testing of existing varieties in order to 1) identify superior varieties for immediate distribution to farmers, 2) furnish foundation material for subsequent improvement, and 3) conserve existing germ plasm for future use. Varieties have now been collected from most of Mexico.

As one might expect, the best open-pollinated varieties were found in the more fertile areas, usually around receding lakes or on lake beds brought into cultivation rather recently. In almost every zone, outstanding open-pollinated varieties were found that could be used extensively with substantial yield increase (20–50 per cent) when chemical fertilizer was added or when planted on otherwise improved soils.

At almost the same time as the distribution of outstanding open-pollinated varieties was started, a program of further improvement through the formation of synthetic varieties was begun as a second step, using the better adapted native varieties as a base.

The first synthetics made and distributed were double top crosses utilizing lines with only 1 generation of inbreeding.

Table 2 shows the results obtained in a study of various top crosses and double top crosses in zone 3. All yields are given in per cent of the open-pollinated variety Urquiza (Urq.). It is of interest to note that in these top crosses and double top crosses germ plasm from 3 different varieties is involved; namely, Leon type I (LI), Tabloncillo (Tab), and Urquiza (Urq.). Leon type I and Urquiza are different varieties of the same race of corn, whereas Tabloncillo is a variety of a different race and a very different type although adapted to similar climatic conditions. It is also clearly evident in the table that top crosses involving the more closely related types (varieties of the same race) are much lower yielding than top crosses between Tabloncillo and Urquiza varieties of 2 distinct races. This agrees with what has been found by corn breeders in the United States. The data show that as a rule one might expect higher yields in crosses of unrelated lines than in crosses of related lines.

Furthermore it is evident in the last column of the table that the yield of the double top crosses is very

TABLE 2.—*Average Yields of Top Crosses and of Double Top Crosses Made from Them*

Cross A	Cross B	Yield of Crosses [a] A	Yield of Crosses [a] B	Average Yield of Top Crosses A + B/2	Actual Yield of Double Top Cross A × B
LI-24 × Urq.	Tab-566 × Urq.	115	155	135	142
LI-162 × Urq.	Tab-566 × Urq.	129	155	142	140
LI-162 × Urq.	Tab-783 × Urq.	129	125	127	135
LI-56 × Urq.	Tab-566 × Urq.	126	155	140	143
LI-24 × Urq.	Tab-395 × Urq.	115	158	136	143

[a] All yields given in per cent of the open-pollinated variety Urq. (Urquiza).

TABLE 3.—*Average Yields of 2 Double Top Crosses Adapted to Zone 4 Compared to the Open-Pollinated Variety Celaya*

Variety	Pedigree	Yield	
		Kgs./Hect.	% of Celaya
H-305	(M-30-60 × Gto. 59A) (C-123 × Jal. 35)	4,845	120
H-301	(C-123 × Gto. 59A) (M-30-33 × Jal. 35)	4,411	108
Celaya	Selected open-pollinated	4,092	100

similar to the average yield of the 2 single top crosses involved. In general, it has been found that the best double top crosses can be made from the best single top crosses.

Although the yield of these double top crosses on the average was about 40 per cent higher than the variety Urquiza, what we need to know is how much of this yield will be held in the advanced generations. The advanced-generation progeny of these crosses yielded on the average 126 per cent of the variety Urquiza. This is a little more than half of what was gained with the first-generation double top crosses. Theoretically one might expect to lose half of what was gained in the first generation. Thus the yield obtained by the synthetic (advanced-generation progeny) is directly related to the yields of the various crosses involved. However, in making up a synthetic variety there are 2 factors working at cross purposes. The greatest yield can be obtained from the intercrossing of unrelated types. But this usually causes trouble when it comes to introduction of the resulting variety and distribution of seed. Wide crosses usually cause so much variability in the resulting product that even the hungriest farmer will discriminate against them. The breeder therefore is limited in what he can effectively use in a synthetic. Also a third factor is involved; this is the disease factor. It has been learned through experience that the varieties of corn found in a specific, isolated region are fairly resistant to diseases prevailing in that region because of natural selection. Although 2 isolated areas may have similar climates, the predominating types of corn may be quite different in their susceptibility to disease. Varieties from region B when tested in region A might give an excellent performance 1 year and practically none the next because of variation in climatic conditions favoring certain disease organisms. Very often, therefore, because of disease, one is limited as to the varieties or types that can be used in the formation of synthetics and hybrids for a specific region.

Synthetic varieties made from double top crosses were introduced with considerable success in zones 2, 3, and 4. They afforded a means of obtaining a small, rapid improvement in yield and certain agronomic characters over the selected open-pollinated varieties. However, in recent years it has been concluded that the most logical breeding program for Mexico is one that combines the use of hybrids and synthetics in a procedure outlined as follows: 1) Wherever possible, make and introduce high-yielding hybrids which in advanced generations will also make good synthetics. 2) Produce and distribute seed of the hybrids only. Increase seed production of the hybrids as demand and technical knowledge increase. 3) Demonstrate continually the value of first-generation hybrid seed over that of advanced generation. 4) Let those farmers who are not able to buy seed each year, or who have not learned the value of new hybrid seed, annually obtain advanced-generation seed from hybrid fields throughout the area and make synthetics through their own natural selection in advanced hybrid generations.

In this way those farmers who understand the use of hybrid seed can obtain the advantages of higher yields, and those farmers who cannot buy seed each year or who do not understand the use of hybrid seed can still obtain significant increases in yield over the best open-pollinated varieties through the use of advanced generations.

Theoretically, the yield of advanced generations of hybrids depends on 3 main factors, as pointed out by Sprague and Jenkins:[3] 1) number of lines involved, 2) average yield of lines, and 3) average yield of all possible single crosses. Which of these factors is most important no one knows, but in the Mexican program we have made very satisfactory hybrids and synthetics from 4 high-yielding, 1-generation selfed lines that combine well in all possible combinations.

The next few tables will help to illustrate the results obtained from double top-cross synthetics and S_1 line double-cross hybrids, which also make good synthetics.

In zone 4, or elevations between 4,500 and 6,000 ft., an excellent open-pollinated variety which has become known as Celaya, from the town of that name, has been widely distributed. It produces fairly good yields on well-fertilized soils.

As in some of the other zones, an attempt was first made to improve the open-pollinated variety Celaya through the production of double top-cross synthetics. The 2 double top crosses that were made and distributed are shown in Table 3.

It is evident that the 2 double top crosses were both superior to Celaya as double top crosses in yield and in agronomic characteristics, but not much superior to Celaya in advanced generation. Hybrid H-301 was about 8 days earlier in maturity and gained considerable popularity from this factor alone.

Two different open-pollinated varieties were used in these top crosses—namely, Gto. 59A, a selection of Celaya, and Jal. 35, an open-pollinated variety from an adjacent region. All S_1 lines were from the Celaya

[3] Sprague, G. F., and M. T. Jenkins. 1943. A comparison of synthetic varieties, multiple crosses, and double crosses in corn. Jour. Amer. Soc. Agron. 35: 137-147.

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TABLE 4.—*Average Yields of Hybrids Made With S_1 and S_2 Lines From Celaya Compared to Yield of the Open-Pollinated Variety Celaya*

Variety	Pedigree	Yield	
		Kgs./Hect.	% of Celaya
H-310	(M-30-60 × C 243-2-2) (Gto. 61-5-4 × Ag. 172-2)	5,359	133
H-309	(C-90 × Ag. 172) (C-123 × C-243)	5,279	129
H-307	(C-67 × C-90) (Ag. 172)	5,084	125
Celaya	Selected open-pollinated	4,092	100

type. The variety Jalisco 35, however, introduced susceptibility to ear rots and root rots, which are quite prevalent throughout zone 4. Celaya carried considerable resistance to both root rot and ear rot, and because resistance to these diseases is an important factor in varietal improvement, we were limited to this type as a source of germ plasm for further improvements in zone 4.

In Table 4 are shown a series of hybrids and double and 3-way crosses, made from S_1 and S_2 lines, all obtained from the variety Celaya.

All hybrids made with S_1 and S_2 lines were substantially superior to the open-pollinated variety in yield and also in ear and root rot resistance.

The first hybrid listed in the table is a double cross made with S_2 lines. It has been more variable in performance from year to year than the second hybrid, made with 4 S_1 lines. The third hybrid listed is a 3-way cross in which the 1-generation selfed line Ag. 172 is very vigorous and an excellent pollinator. Three-way crosses can readily be produced commercially with S_1 lines, and in certain zones some of our best hybrids now being distributed are 3-way crosses. They do not, however, make as good synthetics in advanced generations as 4-line hybrids.

The second double cross (C-90 × Ag. 172) (C-123 × C-243) has been widely distributed under the name H-309 and has become very popular in zone 4. As a double cross it yields about 30 per cent more than Celaya and maintains a 15 to 20 per cent advantage over Celaya in advanced generation as a synthetic. This is better than what we have been able to obtain with the double top-cross synthetics in this zone.

The demand for first-generation seed of this hybrid is steadily increasing. Farmers who plant hybrid seed sell much of their produce as second-generation seed to their neighbors the following year, although they themselves will plant only first-generation seed. With better seed production facilities and organization, the use of first-generation seed will increase rapidly. In the meantime we have accomplished what we have set out to do: to achieve widespread usage of partially improved varieties rather than small-scale usage of excellent hybrids.

In addition to its high yield capacity as a double cross and a synthetic, Celaya has many other advantages. 1) All 4 lines are vigorous with 1 generation of selfing, propagated as open-pollinated varieties. 2) Since the lines are vigorous, seed production is much easier. This is an important factor where the machinery for foundation seed production is not well worked out. 3) The grain on single crosses is large and farmers do not object to planting single-cross seed in double-cross production fields. 4) The 4 vigorous S_1 lines combine well in all combinations and, since all come from the same race of corn, are similar in type. Segregation with respect to type is therefore not very great in advanced generations. 5) The greatest advantage in the formation of hybrids from partially inbred lines is in their flexibility and ease of change as new diseases or new races of the same diseases present themselves. This is a real advantage in Mexico where there are relatively few corn breeders, and it is worth serious consideration in other countries as well.

As a new race of certain disease-producing organisms appears, an S_1 line double cross will at first show little more susceptibility than an open-pollinated variety. For example, in the last few years Helminthosporium blight has become increasingly severe throughout zone 4. When it was first noticed, the 4 S_1 inbred lines were planted on a large scale in an area where the disease was most destructive. Those plants showing the greatest resistance within each line were selfed. The resulting S_2 lines were again subjected to a susceptibility test and the most resistant ones recomposited within each family. Each of the original S_1 lines of the double cross was then substituted with its S_2 composite resistant to Helminthosporium without further modification of the hybrid itself in yield or desirable agronomic characters. Nor has the homozygosity nor vigor of the lines been noticeably changed. By maintaining flexibility the hybrid might be molded in any direction with comparative ease as new races of disease-producing organisms present themselves. Usually when this happens, relatively few genes are involved in resistance and susceptibility and only slight changes in gene frequencies are needed to maintain resistance. In the S_1 line method of hybrid corn breeding, resistance might be maintained with the same relative ease in a hybrid as in an open-pollinated variety.

Our experience has shown greater uniformity in advanced-generation line hybrids, but not greater yield. Our policy in Mexico is to inbreed only to the point where a line can be identified as a line. If 1 generation of inbreeding will do it, we believe no further inbreeding is required or desirable. Further inbreeding, in our opinion, only complicates the situation in maintaining disease resistance.

A general picture of what has been accomplished

TABLE 5.—*Comparative Improvements Obtained in the Various Zones by Different Methods of Breeding With Selected Varieties Taken as 100 Per Cent*

Method of Breeding	Zone 2	Zone 3	Zone 4	Zone 5-6
Common open-pollinated varieties	80–85	75–85	50–80	85
Selected varieties	100	100	100	100
Synthetics	115	126	115	....
S_1 line hybrids	135	141	129	125
Advanced line hybrids	134	140	130	....

in the various zones through the different methods of breeding is in Table 5.

Good, productive, open-pollinated varieties were found in isolated areas on small, fertile, recently-drained lake beds; these were much superior to the common varieties in other areas when planted on fertilized or improved soils. In the table these are taken as 100 per cent and all others are calculated on a relative basis. Through the introduction of these selected open-pollinated varieties a gain of 15–50 per cent was generally obtained over the average of the open-pollinated varieties commonly grown. Another 15–26 per cent has been gained by the formation and introduction of synthetics; and through the formation of hybrids utilizing 1-generation selfed lines, yields have been increased 29–41 per cent more than the selected open-pollinated varieties used as checks. It is interesting to note that, in general, hybrids made with advanced-generation inbred lines from adapted varieties have not yielded more than S_1 line hybrids. However, they are more uniform and somewhat more resistant to the specific diseases prevalent at the time they were developed.

As a result of the introduction of selected open-pollinated varieties, synthetics, and hybrids with superior agronomic characteristics and yield capacity, the Mexican government, by Presidential decree, created the National Corn Commission for the multiplication and distribution of improved corn seed. This organization has accomplished much in the distribution and utilization of improved varieties. At the present time the Commission is the sole agency producing hybrids and synthetics for controlled distribution. The amount of seed distributed during 1953 by this organization is given in Table 6.

TABLE 6.—*Seeds Distributed in 1953 by the National Corn Commission*

Hybrids	Kilograms
Presan (H-1)	144,000
H-123	27,000
Ramillan	354,000
H-309	733,000
H-311	8,000
	1,266,000
Synthetics and Improved Open-Pollinated Varieties	1,000,000

The amount of seed distributed in 1953 is similar to the amounts distributed annually for the last 4 years. From this and from what the farmers distribute themselves as advanced-generation hybrids from year to year, it is estimated that about 15 per cent of the land planted to corn each year is planted with improved seed. What is equally important is that most of this acreage is fertilized with ammonium sulphate and superphosphate.

The present administration in Mexico has placed considerable emphasis on extension work. Already a group of 35 young agronomists with varying amounts of agricultural training have been sent out to the important agricultural areas to help the farmer with his production problems. There is every reason to believe that in the next few years some of the seed production difficulties will be solved and a high percentage of the farmers in each important agricultural region will be growing hybrids with chemical fertilizer.

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COOPERATIVE UNIFORM TESTING OF CORN INBREDS AND HYBRIDS[1]

Merle T. Jenkins[2]

The cooperative uniform tests of corn hybrids were first initiated in 1933 and have been in continuous operation since that time. There were some exchanges and observational plantings of inbred lines during the 1920's and again in the late 1940's. The tests of inbred lines have not been as formally organized, however, as the tests of hybrids. The uniform trials which have been an integral part of our regional corn improvement projects are discussed here.

Corn improvement was organized on a regional basis in 1926 as a result of the action of the Corn Belt section of the conference of experiment station directors held at St. Louis, Missouri, April 21, 1925. The states participating in this sectional conference were Ohio, Indiana, Illinois, Missouri, Kansas, Nebraska, Iowa, Minnesota, Wisconsin, South Dakota, North Dakota and Montana.

Representatives of these states adopted corn improvement as the first problem for investigation under the Purnell funds recently supplied by Congress. At their request a committee was appointed to study the problem and outline the method of investigation. Following the report of this committee the Purnell Corn Improvement Conference was organized and held its first meeting in Washington, D. C., November 17, 1926.

This regional program on corn improvement under the Purnell Act, became the first cooperative regional project for cereal improvement in the United States. It has been extremely successful, as attested by the progress that hybrid corn has made in this country. The Purnell Corn Improvement Conference functioned until 1932 and was followed by the Corn Improvement Conference of the North Central Region organized in 1937 and still in active operation.

In 1939 the Southern Corn Improvement Conference was organized embracing the states of the southeastern region. The Northeastern Corn Improvement Conference, including the states of the northeastern region, was organized in 1945.

The regional approach to crop improvement now is well established in the United States. There are many advantages in this kind of an organization and these advantages have been especially significant in corn improvement. Prior to the organization of the Purnell Corn Improvement Conference, the small, isolated breeding projects of the North Central Region had produced no good commercial hybrids, although some elite inbred lines were available at each breeding station. There was not a sufficient number of elite lines at any station, however, to produce a hybrid worthy of expanded commercial production. Following the interchange of breeding material the elite lines from different experiment stations were combined into hybrids that made hybrid corn a commercial possibility. The same situation exists today. Our best hybrids usually involve elite lines from 2 or more of the cooperating stations within the region.

A similar situation has obtained in the South. There also the early breeding projects were small and isolated and, with the possible exception of Tenn. 10, no outstanding hybrids had been produced. Again it was not until after the organization of a cooperative regional project and interchange of inbred lines among cooperating agencies that good commercial hybrids were produced for the area.

The Food and Agriculture Organization of the United Nations in sponsoring a hybrid corn program for Europe and the Middle East has drawn upon this American experience. The European program has been organized on a regional basis and FAO is doing its utmost to promote a free interchange of breeding materials among workers in the cooperating countries. In furthering the cooperative phases of the project FAO has provided seed for uniform tests of American hybrids and has sponsored 6 international conferences of research workers involved in the cooperative enterprise, which already has shown considerable successful accomplishment.

PURPOSE OF UNIFORM TESTS IN THE REGIONAL PROGRAM.—The importance of the uniform tests in developing effective cooperation in the regional corn projects can not be overemphasized. Their primary contributions have been: 1) developing a suitable "climate" for personal cooperation; 2) providing a wide diversity of testing conditions, thus making it possible to identify strains resistant to a large number of production hazards; 3) promoting the prompt interchange and wide use of outstanding new inbred lines.

The planning and operation of the uniform tests have served an extremely important function in providing opportunity for personal contacts among workers that are essential to effective cooperation, a function equally as important as the data provided by the tests.

The uniform tests usually are scattered rather widely over the region they are intended to sample. For this reason they provide a wider diversity of soil and climatic conditions than would be feasible in any one state. Under such a wide diversity of testing conditions weaknesses such as susceptibility to disease, insects, drought or other conditions are readily brought to light. Outstanding strains which do well under the varied conditions of the regional uniform tests must

[1] Invitational paper presented September 9, 1953, at the Symposium on Cooperative Agricultural Research in the Western Hemisphere, arranged jointly by The American Phytopathological Society, The American Institute of Biological Sciences, and The Potato Association of America, at the 45th Annual Meeting of The American Phytopathological Society in Madison, Wisconsin.

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carry resistance to a rather large number of production hazards. Such strains should aid materially in stabilizing production from year to year.

The uniform tests also have been extremely useful in promoting prompt interchange of outstanding new lines. The information thus supplied has been of sufficient value to breeders to entice them into submitting their best productions for testing. Everyone thus is kept informed of the best material available. Lines entered in the uniform tests are freely exchanged among the cooperating stations for use in their breeding programs. The only restriction usually has been that the lines will not be released to the public without prior approval of the originating station.

ACCOMPLISHMENTS OF THE UNIFORM TESTS.—During the early years of the uniform tests in the North Central region most of the entries in the tests were top crosses for the evaluation of existing inbred lines. The first test of single crosses in all combination was conducted in 1939 and most of the testing has involved single crosses since 1940. Most of these tests of single crosses included all the 45 possible combinations among 10 inbred lines. Data on these crosses then are used to predict the expected performance of the 640 double crosses among these lines. The more promising double-cross combinations then are produced and tested in the individual states. In recent years several of the uniform tests have involved 3-way crosses and the data from them also have been used to predict the expected performance of double crosses.

The first uniform test sponsored by the Southern Corn Improvement Conference was a test of top crosses, conducted in 1942. During the next 4 years the Southern Conference sponsored tests of single crosses among the older lines available at that time. These tests evaluated the inbred lines then available fairly well and the uniform tests were suspended pending the production of new lines. The more promising of these recently developed lines soon will be ready to include in a new series of uniform tests in this region.

The first uniform test sponsored by the Northeastern Corn Improvement Conference was a test of double crosses conducted in 1945. Beginning in 1946 the Northeastern Conference has sponsored uniform tests of single crosses as well as a uniform test of double crosses each season.

In the North Central region one of the first tangible results of the cooperative testing program was the recognition of U.S. 13 as an outstanding combination with a wide range of adaptability. This hybrid probably has been grown on a larger acreage under a wider range of environmental conditions than any other dent corn hybrid. Practically all the outstanding inbred lines which for years have been the backbone of the hybrid corn industry in the Corn Belt were recognized in the uniform tests. Some of the newer elite inbred lines whose value has been emphasized by the uniform tests in this region are Ohio lines Oh07, Oh7A, Oh28, Oh51A, Oh43 and Oh45; Indiana lines H15, H18 and H21; Illinois lines R2 and R38; Iowa lines B8, B10, B11, B14 and B33; Nebraska line N6; Kansas lines K55 and K64; Minnesota lines A71 and A73; Michigan line MS1334; Wisconsin line W22; Connecticut line C103; and Plant Industry Station lines CI.7 and CI.21E. In each case, recognition of the value of the new line was followed immediately by a wide exchange of seed and inclusion of the new line in other programs of the region.

The first substantial results of cooperative tests in the Southern Region was the development of Dixie 17 which involves inbred lines from Tennessee and North Carolina. Dixie 18, involving lines from Louisiana, Florida, and Georgia is perhaps more widely grown than any other hybrid in the South at the present time.

The uniform tests of American double crosses sponsored by FAO have provided the background information for expansion of hybrid corn in several European countries. These tests were initiated in 1948 when FAO distributed seed of 30 hybrids to 19 countries. The tests were designed to acquaint European plant breeders with American hybrids and identify the particular hybrid maturities adapted to the different corn growing regions of Europe and the Middle East. For this reason the 30 selected hybrids involved a wide range of maturities. The tests were continued for 5 years and were terminated in 1952 when it was felt they had completed their particular function. They now are being replaced by uniform tests, involving European breeding material, which are designed to aid in developing hybrids specifically adapted to European conditions. Preliminary tests of top crosses involving European and American inbred lines were conducted in 1951 and 1952. These tests have been expanded during the current season of 1953.

ESSENTIALS OF EFFECTIVE REGIONAL COOPERATION.—On the basis of experience gained in the regional corn improvement programs the following items may be considered essential to the development of a successful regional cooperative enterprise:

1) The work should be organized to promote personal cooperation among all technical workers in the enterprise. Successful cooperation cannot be dictated from above. To be effective it must be personal. Each member of the cooperating team must have respect for the ability and integrity of his fellow workers and be willing to share his knowledge with them. A most essential feature in the development of personal cooperation is to provide facilities for the research workers to meet at frequent intervals, discuss their mutual problems, and become personally acquainted. In our regional corn improvement projects these facilities are provided through our Corn Improvement Conferences which usually meet once each year.

2) Each individual worker and his work must profit from the cooperative undertaking if he is to enter wholeheartedly into the cooperative enterprise. Otherwise he soon will lose interest in the undertaking. The uniform tests provide each research worker with the privilege of entering his outstanding productions in

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the regional tests and obtaining data on their performance under a large number of environmental conditions, many of which he could not provide in his own locality. He also has the privilege of obtaining seed stocks of the outstanding lines developed by other states which perform well in his locality.

3) Some continuing official agency must assume major responsibility for the secretarial work connected with the cooperative undertaking. In the cooperative regional corn improvement projects this facility has been provided by the U. S. Department of Agriculture. We have accepted the responsibility for publishing the reports of the meetings and for tabulating and publishing the results of the uniform tests. If there is to be continuing interest in uniform tests the data from them must be analyzed and made available promptly to all cooperators.

A continuing official sponsor is even more imperatively essential if the regional cooperation is international in character. In the regional corn improvement project organized in Europe and Middle East, FAO has sponsored the meetings and has summarized and published the results of the uniform tests. Without the aid of FAO the cooperative phases of this work would promptly collapse.

In conclusion I may say that I believe our uniform tests of corn hybrids and inbred lines have been a very effective part of our regional corn improvement work. They should be viewed, however, as one part of the over-all regional project and not as an end in themselves.

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MEXICAN WHEAT PRODUCTION AND ITS ROLE IN THE EPIDEMIOLOGY OF STEM RUST IN NORTH AMERICA[1]

Norman E. Borlaug[2]

SUMMARY

Despite the set-backs which have been caused by changes in races, a great deal of progress has been made since the wheat improvement program of the Oficina de Estudios Especiales was established in 1943. Yields per acre are increasing, new areas suitable to economical wheat culture have been established, and the 1953 harvest is the largest in Mexican history. Much of the most important basic agronomic information has been obtained and much of it is being successfully put into commercial application. The ever-changing variable, the rust population, is one of the limiting factors in wheat production that has not been solved permanently. Nor is it likely to be solved in the future, for the variability of this type of pathogen is extremely great and will continue to require research to meet the constantly changing race composition. More flexibility in approach than that which is possible by conventional breeding methods is necessary and is being investigated. This truly international problem is not restricted to stem rust, for in Mexico, at elevations above 7500 ft., the stripe rust problem is also imposing. In some other parts of the hemisphere, leaf rust is more important than either of these other 2 rusts of wheat.

In considering the international wheat stem rust problem in North America, most farmers must reorient their thinking with respect to the value of severe winters and high summer temperature in reducing rust inoculum. Both of these factors often contribute to a bountiful wheat crop in the forthcoming year, and consequently are a blessing in disguise.

American farmers have obtained great benefits from the use of stem rust-resistant varieties during the past 20 years. The success of these varieties has been due at least in part to the "escape mechanism" of disease control. The use of this mechanism will be even more important to the farmers of the northern Great Plains in the next few years until the newer varieties with resistance to race 15B and others become generally available. In a mountainous country such as Mexico, winter temperatures are not sufficiently low nor the summer temperatures sufficiently high to destroy urediospores of the stem rust fungus. Therefore, over the centuries the farmer has become fully aware of the value of the "escape mechanism" as the surest means of obtaining a successful crop. He has learned to manipulate dates of planting and to use irrigation water properly to minimize the losses from rust. In considering the role of the Mexican wheat crop in the epidemiology of the stem rust organism (*Puccinia graminis tritici*) in North America, nearly equal attention must be given to the Mexican barley crop

[1] Invitational paper presented September 9, 1953, at the Symposium on Cooperative Agricultural Research in the Western Hemisphere, arranged jointly by The American Phytopathological Society, The American Institute of Biological Sciences, and The Potato Association of America, at the 45th Annual Meeting of The American Phytopathological Society in Madison, Wisconsin.

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which is also a constant reservoir of inoculum.

CONDITIONS UNDER WHICH WHEAT IS GROWN IN MEXICO.—Wheat is grown commercially from 32° along northern border to 16° in the high valleys of Chiapas in the South. Within this zone wheat is grown from a few feet above sea level in Sonora to an elevation of 10,000 ft. in some of the high valleys of South Central Mexico. More than 95 per cent of the wheat crop is grown either under irrigation or on reserve soil moisture during the winter months. Plantings are generally made during the months of October, November, and December, and harvesting is done during April, May, June, and July, depending on the elevation. Planting dates must be manipulated so that the wheat does not head until the danger from frosts has passed. All varieties are of spring habit (7, 2, 14).

The relatively small acreage devoted to the summer culture of wheat is planted in May and June and harvested in October and November (8). Virtually all the barley is planted and harvested during this same summer period. The summer plantings of both wheat and barley are not irrigated and the dates of planting coincide with the beginning of the summer rainy season. Harvesting is done at the beginning of the winter dry season.

SMALL GRAIN PRODUCTION REGIONS IN MEXICO.—The areas devoted to wheat and barley production can be divided into 4 principal regions on the basis of production and breeding problems.

Region #1—The Pacific coastal plain (15–300 ft. elevation).—This region comprises the irrigated coastal plain of Sonora and, more recently, it has extended southward into Sinaloa. Irrigation water is obtained from large dams and in some cases from deep wells. The cultivated lands in this region are relatively new, none having been cultivated more than 40 years. Large areas of new land are being opened to culture as new irrigation projects are completed. Yields on new lands are high, but diminish rapidly after 5 years in cultivation unless fertilized properly. Lodging, shattering, and control of stem rust are important considerations in this region. Wheat culture has extended rapidly southward into Sinaloa in the past 2 years. This western coastal region at present produces 50 per cent of the wheat crop of the Republic. All the land preparation, planting, and harvesting are done by modern machinery. Cotton and rice are the principal summer crops in this region.

Region #2—La Laguna (3000 ft. elevation).—This region comprises a very specialized region in Northern Mexico. Soils are for the most part highly alkaline and of low fertility. The atmosphere is extremely dry and rusts are not a serious problem. Irrigation water is obtained from dams and deep wells. The planting and harvesting are done largely by machinery. Cotton is the principal summer crop in this area.

Region #3—El Bajio (5000–6500 ft. elevation).—This region comprises the black soils of Central Mexico. They have been cultivated continuously since colonial times and are now of extremely low productivity unless properly fertilized. Irrigation is provided from dams and deep wells. Stem rust sometimes causes severe losses. Mechanization within this region has increased greatly within the past 5 years, but much of the area is still cultivated by primitive methods. Corn is the main summer crop.

Region #4—The mountain valleys (6500–9500 ft. elevation).—This region is made up of a heterogeneous group of scattered high mountain valleys, extending from Northern to Southern Mexico. Soils are of low fertility. Irrigation water is provided by small dams, deep wells, and in some cases wheat is grown on reserve soil moisture. Stem rust may become destructive in some years throughout this entire area. Stripe rust is also a problem. Virtually all production within this region is carried on by primitive methods.

It is within this region that virtually all the barley and the summer wheat are grown. Corn is the most important additional summer crop in this region.

RUST EPIDEMIOLOGICAL CLASSIFICATION.—On the basis of consideration of the epidemiology of the stem rust organism, Mexico can be divided into 2 zones: 1) the Pacific Coastal Zone and 2) the Mountain Zone.

The Pacific coastal zone (10–500 ft. elevation).—Summer temperatures are extremely high in this area and summer rains are unpredictable. The mode of over-summering of stem rust urediospores in this region is unknown. Although occasional volunteer wheat plants emerge and develop poorly, stem rust has not been found on these volunteer plants. Perhaps infected volunteer plants survive in protected areas along irrigation canals, or perhaps the rust survives in the uredial stage on grasses or barley in the mountains, and the spores are blown back down to the coast in the fall. The first infection of stem rust on wheat generally is not found on the west coast until January.

Mountain zone.—At elevations of 4000 ft. and above, the stem rust organism frequently survives in the uredial stage throughout the year. Rusted volunteer wheat plants are common in the corn and bean fields during the summer. Barley and wheat are cultivated during the rainy season in many valleys above 7000 ft. and generally these become infected with rust. Several species of grasses, especially wild barley, have been found infected with stem rust. Clumps of *Hordeum jubatum* have been observed in the valley of Mexico where the uredial stage of stem rust has survived continuously over a period of 3 years. Similarly, at Chapingo the uredial stage has been observed to survive on 3 generations of volunteer wheat plants over a period of 18 months.

Throughout this zone the amount of inoculum of stem rust is reduced greatly at the onset of the dry season in October and November as a result of the dryness of the atmosphere and by repeated light frosts.

RACES OF PUCCINIA GRAMINIS TRITICI IN MEXICO.—The early investigations of Stakman and coworkers

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established that the races of the stem rust pathogen prevalent in Northern Mexico generally were the same races that were prevalent during a similar period in the Great Plains of the United States and Canada. These early studies also indicated that it was several years after a race of rust became established in Northern Mexico before it appeared in South Central Mexico, indicating that some isolating factor influenced the interchange of inoculum between these 2 regions.

When the wheat improvement program of the Oficina de Estudios Especiales was begun in 1943, races 17, 19, 38, 56, and 59 were prevalent in 1 or more geographic areas of Mexico (15). Therefore, the first step was to obtain suitable varieties with resistance to these races.

IMPROVEMENT PROGRAM OF THE OFICINA DE ESTUDIOS ESPECIALES.—Prior to 1943, virtually all the area planted to wheat was seeded with "native" varieties of unknown origin. The only varieties of known origin were Mentana, which was introduced from Italy in the late 1920's, and a small acreage in Sonora which was planted with Ramona and Baart from California. Virtually all the "native" varieties, with the exception of Aguilera, Candeal, and Barrigon, were badly mixed, and most farmers' fields were mixtures of many different types. It was common to encounter 10–20 morphologically different types of wheats, belonging to 3 different species, in a single field. These types differed greatly in maturity, height, resistance to lodging, shattering, and in grain texture, resulting in complicated harvesting, milling, and baking procedures (2, 7).

From the time the program was initiated, the problem was attacked from 3 different points of view, namely: 1) improvement of cultural practices, 2) studies to determine proper fertilization and rotation practices, and 3) a breeding program to develop disease resistant varieties for each region, suited to improved cultural and fertilizer practices.

The breeding program from the beginning was based on 3 different approaches: 1) selection in "native" varieties, 2) introduction and reselection in materials from other countries, and 3) a crossing program, wherein the best "native" types were crossed with the best introductions (2, 14).

IMPROVEMENT THROUGH SELECTION.—Approximately 8500 individual head selections were made in farmers' fields in different parts of the Republic in 1944 and 1945. Within this group more than 5000 selections were of bread wheat types. All but 2 of these proved to be highly susceptible to stem rust in subsequent tests. The 2 lines with considerable resistance were extremely long-strawed and susceptible to lodging and, consequently, were not used as commercial varieties. However, they were used extensively in the breeding program. Of the remaining 3500 selections which were largely representatives of the species *Triticum durum* and *T. turgidum* ("hump-backs"), many were found to be resistant to the races of stem rust then present. However, Mexico's need was one of increasing the bread wheat acreage and reducing the acreage cultivated to "hump-backs" and durum wheats. Therefore, none of these latter wheats was multiplied for commercial usage. In 1944 probably 50 per cent of the entire area cultivated to wheat in the Republic was of the "hump-back", and, to a lesser extent, durum types. The large area cultivated to "hump-backs" was the result of the disastrous stem rust epidemics on bread wheats during 1938 and 1939. The 2 "hump-back" varieties were highly resistant to the common races of the stem rust pathogen that were then prevalent.

During the first 3 years of the program particular attention was focused on the introduced materials. From this material 4 varieties were developed by reselection. Two of these, Supremo 211 and Frontera, came from reselections made in hybrid material received from E. S. McFadden of Texas, and the remaining 2 were reselections from Kenya wheats. These varieties were distributed commercially in 1948 and served a useful purpose through 1951 and 1952 by which time they were largely replaced by the varieties Yaqui 48, Chapingo 48, and Kentana 48, which were developed from the breeding program begun in April of 1945. The former 2 varieties were derived from a cross of Marroqui (originally a Moroccan variety) × Newthatch, and the latter from a cross of Kenya × Mentana. Lerma 50, which was derived from the second backcross of Kentana to Mentana, was released in 1951, and Gabo, a variety "screened" from the U. S. Department of Agriculture world wheat collection, was first increased in 1951.

Since the initiation of the breeding program in 1945, 2 field generations of all crosses have been grown each year. The winter planting in November and December which simulates the planting of the main commercial crop is made at 7 regional nurseries varying in elevation from 20–8500 ft. in widely dispersed geographic areas of the Republic. All lines selected from the winter nurseries are regrown in 1 of the 4 summer nurseries which are planted during May and June. Selections in the summer nurseries are made primarily on the basis of resistance to stem and stripe rusts. Severe epidemics have developed in the summer nurseries since the program was initiated, thereby greatly facilitating the selection of lines resistant to stem rust. During the first 6 years of the program all selections for disease resistance were made on the basis of field reaction alone. For the past 2 years satisfactory greenhouse facilities have become available and are used for checking the seedling and adult plant reaction of all lines which enter regional rod-row trials.

Special effort was made to incorporate a broad base of resistance to stem rust. Large numbers of crosses have been made and processed. All the material for the first 6 years was handled using the Pedigree method. Since 1951 major emphasis has been placed on further improvement through use of the backcross

TABLE 1.—*Shift in varieties grown in El Bajio (#3) and mountain valley (#4) regions from 1948 to 1953*

Variety	Percentage of Commercial Average		
	1948	1950	1953
Native Varieties			
1. "Barrigon" ("Hump-back")	30	30	15
2. Old Bread-Wheat Varieties (Rojo, Candeal, Queretaro, Mentana, etc.)	60	30	10
Improved Bread-Wheat Varieties			
1. Supremo 211	5	30	5
2. Kenya	4.5	5	0
3. Kentana	0.5	5	50
4. Lerma	0	Trace	20

method since the improved varieties Yaqui, Gabo, Lerma and Kentana possess most of the desired characteristics.

RESULTS FROM BREEDING PROGRAM.—The rapid acceptance of the first 4 varieties of wheat derived from the breeding program, and of Gabo, which was screened from the U.S.D.A. World Wheat collection, is indicated in Tables 1 and 2 for the Bajio and High Valley and Pacific coastal regions, the 2 most important wheat producing areas in the country. It is estimated that the new varieties which were in semi-commercial increase in 1948, were planted on more than 70 per cent of the area cultivated to wheat in 1953. Once the new varieties were released they were multiplied twice each year, thereby resulting in large volumes of seed being made available to the farmers in a short time. The rapid acceptance of these varieties is the result of a combination of characteristics. They were more resistant to the prevalent races of stem rust than the native varieties which they replaced except in the case of the hump-back "Barrigon". Moreover, these newer varieties were better adapted to an improved agriculture. Heavy fertilization could be practiced without danger of lodging, whereas all the native varieties lodge badly when fertilized heavily. The new varieties were well adapted to combine harvesting which has become common in many areas of the Republic within the past few years. The newer varieties were from 10 to 18 days earlier than the varieties they replaced, thereby making it possible to grow a larger acreage of wheat with a given quantity of water for irrigation. The milling and baking properties of the new varieties were superior to the former varieties. The sum total of these advantages has resulted in much higher yields per acre to farmers who combine the use of the improved varieties with improved land preparation, proper fertilization, and proper use of irrigation water. The best farmers in the Bajio area, on land which has been farmed continuously for 350 to 400 years, are now harvesting 45 to 60 bu. per acre on the same land where they were harvesting 8 to 15 bu. per acre with old varieties and old methods only 5 years ago. The outstanding example of progress in increasing wheat production on a given tract of land is that of a Sonora farmer who this year harvested 5600 bu. of Gabo on the same 80-acre tract where he harvested 640 bu. of Aguilera 3 years ago. This phenomenal increase in yield is not to be expected on all farms. However, by combining proper cultural, fertilization, and irrigation practices with the use of the proper improved variety, it should be possible to double the national average yield within the next 5 years, providing stem rust can be kept under control. The newly organized extension service, backed by sufficient agricultural credit, can put the improved practices into operation on the *ejidal* farms.

TABLE 2.—*Shift in varieties in Pacific coastal plain (#1) region from 1948 to 1953*

Variety	Percentage of Commercial Average		
	1948	1950	1953
Native Varieties			
1. Barrigon ("Hump-back")	75	60	15
2. Old Bread-Wheat Varieties (Aguilera, Candeal, Baart, Ramona, etc.)	25	10	Trace
Improved Bread-Wheat Varieties			
1. Kenya	0	5	Trace
2. Yaqui "48"	Trace	20	65
3. Kentana "48"	0	5	10
4. Lerma "50"	0	Trace	4
5. Gabo	0	Trace	4
6. Others	----	----	2

With the development of early-maturing stem and stripe rust-resistant varieties and with the use of chemical weedkillers, it has become economically feasible for the first time to grow wheat during the summer rainy season in the high valleys of South Central Mexico.

SHIFT IN RACES OF STEM RUST.—Prior to 1951, races 17, 19, 38, 56 and 59 of *Puccinia graminis tritici* were prevalent in Mexico. The 5 improved varieties described earlier are resistant or moderately resistant to all these races. Race 15B became present in the Northern Great Plains of the United States and Canada in the summer of 1950. Prior to that time it had never been found in Mexico. By April and May of 1951 it began to appear in widely different geographic areas of the Republic, from Sonora on the Pacific coastal plain to the states of Puebla, Mexico, Michoacan and Jalisco in South Central Mexico. By September of 1951 this race constituted 70 per cent of the isolates in Central Mexico. Of the new varieties, Yaqui, Chapingo, and Gabo proved to be susceptible to race 15B, whereas Kentana and Lerma, with Kenya type of resistance, were found to be resistant. The screening effect of these latter 2 varieties is clearly indicated by Fig. 1, which shows that the prevalence of race 15B in North and South Central Mexico rapidly decreased during 1952 and 1953 as the acreage of Kentana and Lerma increased. On the Pacific coast where Yaqui, susceptible to 15B,

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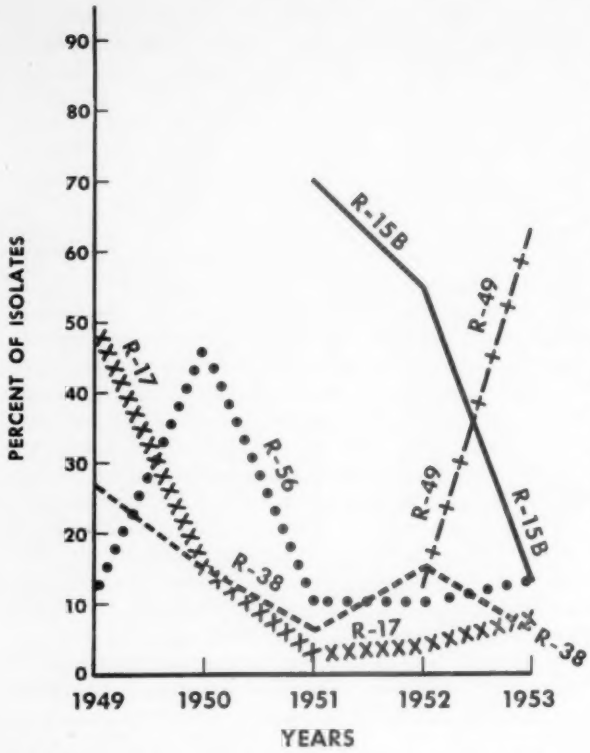

FIG. 1. Relative prevalence of physiologic races of *Puccinia graminis tritici* in the Bajio and High Valley regions (#3 and 4) from 1949 to 1953. (Note effect of varieties on prevalence of races 15B and 49 in Fig. 1 and Fig. 2.)

comprises the major acreage, race 15B constitutes more than 60 per cent of the isolates (Fig. 2).

It is of interest that race 15B has not yet reached the wheat-growing areas of Guatemala. Supremo grown there is extremely susceptible to race 15B but has developed no rust through 1952.

Almost concurrently with the rapid decrease in race 15B in Central Mexico, where a large percentage of the area is Kentana and Lerma, there has been a very rapid build-up of 2 new races, namely 49 and 139. The former race was common in the United States and Northen Mexico in the 1930's and decreased or practically disappeared by 1943 as the Hope type of resistance became common in the United States and Canadian wheat belts. It was not found in South Central Mexico prior to 1953.

Race 139, which from the breeders' standpoint is very similar to race 49, had been isolated several times prior to 1950 but had never shown signs of becoming prevalent. It is now known that these 2 closely related races began to increase on late planted fields of Kenya 324 in the Sierra de Arteaga near Saltillo, Coahuila, in the spring and summer of 1950. Since no commercial damage was caused, the outbreak was unreported. The race increased last summer in the same area and by July had killed several thousand acres of Kenya 324. During September of 1952 many infection centers of race 49 began to appear in South Central Mexico on Lerma and Kentana, which carry Kenya type of resistance to race 15B and are susceptible to race 49. The present indications are that this race will constitute more than 90 per cent of the isolates in the states of Hidalgo, Mexico, and Puebla this summer. The varieties Yaqui and Gabo, which are susceptible to race 15B, are highly resistant to races 49 and 139 (6, 9, 10).

PROBLEM FOR THE IMMEDIATE FUTURE.—As a result of the 2 sudden shifts in the stem rust population within the past 3 years, none of the 5 improved commercial varieties possesses resistance to all widespread races. Once again we must adhere strictly to the escape mechansim of proper dates of planting and proper manipulation of irrigation schedules to minimize the hazard of stem rust.

At the present we are beginning to increase 3 new varieties which apparently carry combined resistance to races 15B, 49, 139 and to the 5 formerly common races 17, 38, 56, 19 and 59 (6, 10). The question is how long will these varieties remain resistant? Will they remain resistant over a period of 15 years

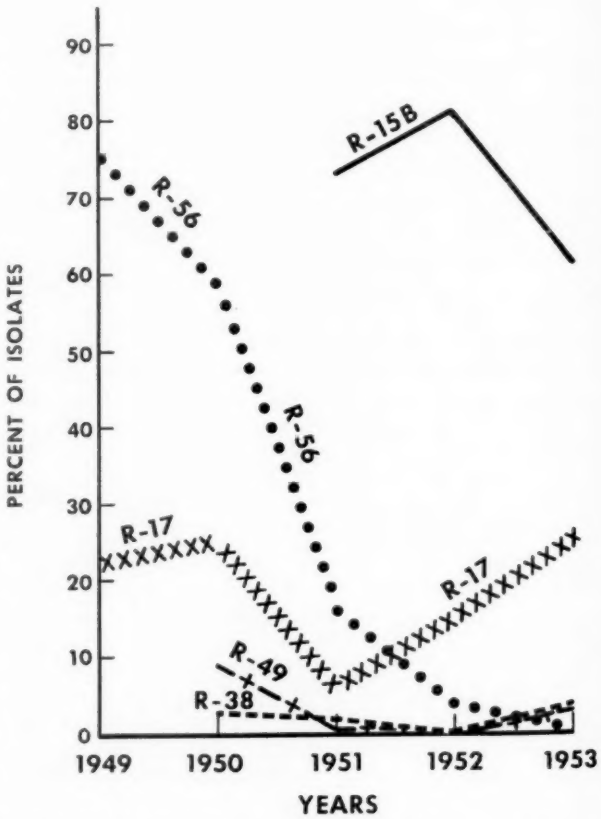

FIG. 2. Relative prevalence of physiologic races of *Puccinia graminis tritici* in the Pacific Coastal Plain from 1949-1953.

as did Thatcher in the United States and Canada and as did Barrigon, the "hump-back" variety of the coastal plain of Sonora, or will they become susceptible through a shift in races within 1 to 5 years as did Lee and Kentana? No one can safely predict the answer to these questions. Nevertheless several points have become increasingly clear within the past few years with respect to the epidemiology and breeding problems, namely:

1) In the autumn the rapid, long-range dissemination of spores of new races from Northern United States and Canada to South Central Mexico can and does take place in 1 step, not necessarily by several, and in the spring the northward movement of spores occurs in a similar manner.
2) There seems to be no barrier to the exchange of spores from Northern Mexico to South Central Mexico.
3) The value of the escape mechansims as an indirect means of control of stem rust and of extending the period of usefulness of a resistant variety in the Great Plains of North America cannot be fully appreciated until one has had an opportunity to observe the potentially rapid increase of inoculum under conditions where the parasite survives perpetually in the uredial stage.
4) Improved agricultural practices often increase the rust hazard by changing the micro-climate within the grain field. Fields grown under old methods, poor seed bed preparation, poor stands, no fertilization, and yielding 8 bu. per acre generally have only 50 heads per square meter, whereas a field heavily fertilized, with a good stand of plants yielding 70 bu. per acre on the same soil type, will have from 500 to 530 heads per square meter. In the low yielding plot the dew will dry off the plants by 9:30 A.M., whereas in the high yielding plot the plants will still be wet at 1:30 P.M.
5) The hazard of stem rust in Mexico may increase as the culture of wheat spreads farther south on the Pacific coastal plain. With the establishment of the new "Falcon" International Irrigation Project on the lower Rio Grande, wheat will undoubtedly become one of the important winter crops on the Mexican side of the river. This may result in the inoculum moving into the Great Plains somewhat earlier than it does at present.
6) There is an urgent need for modification of the present stem rust differentials, so that "gene testers" and varieties which are used currently in breeding programs will be incorporated into the differentials used in the identification of the rust population. This will bring about a closer and more direct coordination between rust population studies and breeding programs.
7) There is need for a new approach to the breeding problem. The use of "flexible composite synthetic varieties," which are made up of phenotypically similar lines that are genotypically different for resistance, is proposed and being developed by our organization. The lines employed in such a variety would be bulit up by the conventional backcross method but would be mechanically mixed to form the composite variety. Then the composition of the variety can be modified rapidly as the rust population shifts (3).
8) There is urgent need for extensive research in an attempt to develop a satisfactory chemical control for rust. This control could be used in any emergency when epidemics are imminent and when shifts in the rust populations temporarily render all or most varieties susceptible. Very little effort and research have been directed toward this end.
9) The cooperative International Nurseries which have been in operation the past 3 years have developed a wealth of valuable information which is basic to all breeding programs. It is certainly one of the outstanding achievements in international technical cooperative efforts (1, 4, 5, 11, 12, 13).

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PROGRESS IN THE CONTROL OF WHEAT[1] STEM RUST IN CANADA

W. F. Hanna[2]

Stem rust was first reported on wheat in Western Canada in 1891. By 1916 it had become a limiting factor in wheat production, destroying in that year 100 million bushels. The average annual loss from 1916 to 1936, when stem rust resistant varieties were introduced, was approximately 35 million bushels.

Canadian rust investigations have been centered at Winnipeg, Manitoba, in the Rust Research Laboratory of the Canada Department of Agriculture. This laboratory was established in 1925 as a result of a decision made the preceding year at a conference of Canadian and United States scientists. During the 28 years the laboratory has been in existence investigators there have cooperated closely with their colleagues in the United States. Progress in the control of cereal rusts in Canada and the United States as a result of coordinated effort and the exchange of information and materials emphasizes the value and importance of international cooperation in scientific research.

At the time investigations at Winnipeg commenced, the life history of the stem rust fungus had not been fully worked out and the principal source of infection in Western Canada was still undetermined. Barberry had been introduced in Western Canada to only a limited extent and by 1925 practically all plantings, mostly in cities and towns, had been located and destroyed. But stem rust continued to appear every year, sometimes in epidemic proportions. Stakman at Minnesota had shown that there were many parasitic strains or races of wheat stem rust. Already 17 races had been identified in Canada. Biffin in England had discovered that rust resistance is a heritable character, and scientists at the University of Minnesota had made considerable progress in breeding stem rust resistant wheats. This briefly was the state of knowledge about wheat stem rust when the Canadian scientists began their program of investigations.

Investigations carried out over the past 28 years have yielded much new information about wheat stem rust, and notable progress has been made in the breeding of resistant varieties.

It has been established that the urediospores or summer spores of stem rust rarely, if ever, overwinter in Western Canada. The rust spreads northward each spring from Mexico and the southern States where urediospores are able to survive the winter, and reaches southern Manitoba by late June each year. From there it extends northward and westward into Saskatchewan.

Seventy-five races and biotypes of wheat stem rust have been found in Canada. Most of these races have been found also in the United States. Certain races have tended to predominate, and have been present over a wide area; others have occurred sparsely and have had a restricted distribution. The most common races have been 11, 17, 21, 29, 36, 38, and 56. Race 56 was responsible for the severe rust epidemic of 1935 which caused such heavy damage to the variety Ceres in Manitoba. In 1950 race 15B, which was found in Western Canada for the first time in 1945,

[1] Invitational paper presented September 9, 1953, at the Symposium on Cooperative Agricultural Research in the Western Hemisphere, arranged jointly by The American Phytopathological Society, The American Institute of Biological Sciences, and The Potato Association of America, at the 45th Annual Meeting of The America Phytopathological Society in Madison, Wisconsin.

Contribution No. 1327 from the Botany and Plant Pathology Division, Science Service, Department of Agriculture, Ottawa, Canada.

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became widely distributed. It has now superseded race 56 as the predominant race. All varieties of wheat grown in Western Canada are susceptible to 15B. Thus far the hard red spring wheats have escaped appreciable loss but the durums have been severely rusted. Three distinct strains of race 15B have been differentiated at the Winnipeg laboratory—15B-1, 15B-2, and 15B-3.

Craigie's classic researches on the sexual process of the stem rust fungus proved to be of great fundamental and practical significance. His discovery opened the way for a series of experiments on the genetics of the cereal rusts. These experiments revealed that the potentialities for variability in the stem rust fungus are almost unlimited. Thus some races were found to be homozygous, others were heterozygous; it was shown that varieties and races of stem rust may hybridize when passing through the sexual stage on the barberry and give rise to new races; abnormalities in spore color appeared; urediospores and teliospores were sometimes formed on the barberry although they normally occur only on wheat, other cereals, and grasses; pycnia characteristic of the sexual stage on barberry were found on wheat. All this work revealed the common barberry in an entirely new role as a potent source of new races of stem rust.

A program of breeding for rust resistance was begun in 1925. The difficulty of obtaining seedling as well as mature plant resistance to all the prevalent races of stem rust seemed, for a time, to be insurmountable. Then it was discovered that certain wheats were resistant in the mature plant stage even though susceptible as seedlings. As wheat in Western Canada is normally well advanced towards heading by the time stem rust infection becomes common, the mature plant type of resistance appeared to be adequate. The variety Renown, released in 1936, and the varieties Regent and Redman, released later on, possess this type of resistance.

The limitations of mature plant resistance became apparent when it was found that wheats possessing this type of resistance are susceptible to race 15B in both the seedling and adult plant stages. Thatcher, produced at the University of Minnesota and introduced into Western Canada, is also susceptible to 15B in both stages of growth. That breeding for rust resistance is an exceedingly difficult and complex problem is illustrated by the recent discovery that C.T. 186, a promising new variety possessing resistance to 15B, is susceptible to a strain of this race—15B-3.

Although efforts to control stem rust have centered around the breeding program, chemical methods of control have not been overlooked. Extensive experiments conducted between 1925 and 1930 demonstrated that satisfactory rust control could be obtained by the application of sulphur dust. During the past 3 years there has been a revival of interest in chemical methods of control in view of the heavy losses caused to durum wheat by race 15B and the possibility that the hard red spring wheats may suffer similar damage.

Rust research has proved to be a highly successful and profitable undertaking. The growing of resistant varieties in Western Canada for the past 17 years has saved many hundreds of millions of bushels of wheat from destruction by stem rust. But today the rust problem appears to be more difficult and complex than when investigations began. Altogether, more than 200 races of wheat stem rust have been identified in various countries where wheat is grown and it now appears probable that there are in existence many times that number. The stem rust fungus has a vastly greater capacity for variability than was formerly suspected. The process of variation which investigators have been observing and studying in the laboratory has been going on for many years, and in all probability has resulted in an immense accumulation of races, many of which have not as yet been encountered. Additional races are continually being produced on the barberry. As new varieties of wheat are introduced, rare or unknown races that can attack them tend to increase and become predominant, just as 15B did in 1950. To expect that present commerical varieties of wheat will continue to remain resistant would be an unwarranted assumption. Experimental evidence and experience in Canada, the United States, Mexico, Kenya, and Australia suggest that they will be attacked by new races of rust.

Solution of the rust problem will require more research. A systematic search will have to be made for new breeding material; otherwise the present stock may soon be depleted. More information about the nature of rust resistance is required—why certain varieties are resistant and others susceptible. Breeding programs will have to be expanded and facilities provided to expedite the testing of material in the greenhouse and the field. Genetic studies on the inheritance of rust reaction offer promise of providing a broader basis for rust resistance. The possibility of utilizing crosses between certain wheat species and wheat relatives is already being explored.

The control of wheat stem rust has become an international problem. Canada, the United States, Mexico, and a number of Latin American countries are already cooperating in testing breeding material and studying rust races. There are many advantages to be gained from having this cooperative work extended to other wheat growing countries where stem rust occurs.

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LATE BLIGHT IN MEXICO AND ITS IMPLICATIONS[1]

John S. Niederhauser,[2] Javier Cervantes,[3] and Leopoldo Servín[3]

A little more than a century ago, a few scientists interested in the control of a new and ravaging blight of potatoes made their first observations on the causal fungus and the disease. Since then, many plant scientists have devoted their efforts to the study of this disease, and late blight has been recognized as a world-wide problem in the production of this basic food crop. Farmers in all parts of the world have adopted protective sprays as a standard practice to prevent disastrous outbreaks of the disease.

During this century of study and struggle with *Phytophthora infestans* (Mont.) DBy., farmers and scientists alike have been intrigued by the possibility of using resistance to combat the blight. They have been searching for a variety that is resistant in the field, in order to avoid or to reduce the expense and inconvenience involved in spraying. The history of this search for resistance tells of a succession of "resistant" varieties that soon become susceptible. We now know that a number of different races of *P. infestans* exist; this knowledge serves us well in that we can explain more logically the past failures and can understand more precisely the difficulties that confront us. It is becoming more and more apparent that plant pathogens are not restricted by national boundaries, and that research done in one country is useful and suggestive to workers elsewhere.

In Mexico, potatoes are grown principally when and where late blight is not likely to be a problem. The grower in the mountains of Puebla in Central Mexico plants his potatoes in late January, during the dry season, hoping for just enough rain to grow a crop but not enough to favor blight development. The León farmer grows his crop in the dry season under irrigation. Thus late blight is escaped, rather than controlled. Only recently have potatoes been planted during the summer rainy season. Where proper equipment and fungicides have been used, the yields have been spectacular and potato growing at this time of the year has proved thoroughly successful. In Mexico the potato is not a staple food and is still somewhat of a luxury. The only way it can be made an important food there is to have a blight-resistant potato that will give a crop during the rainy season, with neither irrigation nor fungicides.

Most, if not all, of the wild species of *Solanum* that are highly resistant to late blight are found in Central Mexico. One species in particular, *Solanum demissum* Lindl., has been widely used as a source of resistance to late blight, and it has provided the resistance genes now being exploited in all of the more important potato breeding programs in Europe and North America. Until recently, what was known about the resistance of these wild species to *P. infestans* was limited to the results of exposing selected clones to physiologic races of the pathogen isolated in Europe, Canada, and the United States. In 1952 it was shown that most of these *Solanum* species, some of which had been reported to be immune, are susceptible to several of the naturally occurring races of *P. infestans* in Central Mexico.[4] It is particularly important to know more about the resistance of these wild species in their native habitat. The level of resistance that they have attained here after a long coexistence with *P. infestans* may indicate the ultimate level of resistance that we can hope for in a potato breeding program.

Little attention has been given to the races of *P. infestans* prevalent in Central Mexico where the resistant wild species abound. Clones of these species, which have survived an annual exposure to *P. infestans* over a long period of time, have provided the necessary suscepts for the selection and propagation of new races of the pathogen. These races might be expected to be highly specialized, and such has proved to be the case.[5]

Mexico is a unique location insofar as the prevalence of specialized races of *P. infestans* is concerned. Some of these specialized races have been found in breeding plots in other parts of the world, but in Central Mexico the highly specialized races are among the prevalent field races. In other words, the race picture in Central Mexico is a preview of what can be expected in other places where and when resistant selections are made and increased for commercial production.

It is in this environment that we are conducting our field trials; it is for this population of races that we are trying to breed late-blight resistance; and it is for this area that we hope to produce a commercially acceptable potato that will need little or no spraying for the control of late blight.

In 1948 several named varieties developed by Dr. Reddick at Cornell University were planted in the

[1] Invitational paper presented September 9, 1953, at the Symposium on Cooperative Agricultural Research in the Western Hemisphere, arranged jointly by The American Phytopathological Society, The American Institute of Biological Sciences, and The Potato Association of America, at the 45th Annual Meeting of The American Phytopathological Society in Madison, Wisconsin.

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Valley of Mexico, where they promptly blighted and died. This was the first evidence of the prevalence of specialized races of *P. infestans* in Mexico. We now know that these plants carried the single major gene for resistance to late blight that Drs. Reddick, Mills, and Peterson have designated as "D" and that Dr. Black has called "R_1". This gene gives resistance only to race 1 of the pathogen.

In 1949 and 1951 other varieties, including Kennebec, were tried and met a similar fate. In 1952 Dr. Mills brought to Mexico 143 clones with the "CD" genes for resistance (Black's "R_1R_2"), which up to that time had been resistant to all races tested in the United States. When planted in the field in the Valley of Toluca, near Mexico City, they all developed blight and most were killed outright. But a marked variation in susceptibility was noted, and "tolerant" or "partially resistant" selections were kept for further trials.

In 1953 nearly 4,000 lines were tested in the field near Toluca. Most of these lines were furnished by Dr. F. J. Stevenson of the United States Department of Agriculture, and a lesser number by Drs. Krantz and Eide of the University of Minnesota. These lines either had survived the blight screening given them in the U.S.D.A. trials or had shown some level of resistance at Minnesota. Only 6 weeks after planting, 94 per cent of these seedlings had been severely blighted or killed. The other 6 per cent showed varying degrees of resistance. Perhaps it is significant that the isolates of the pathogen from these resistant plants were not always the most highly specialized races.

In the course of making these field observations on resistant seedling lines, about 90 cultures of *P. infestans* from a wide variety of sources have been isolated. A considerable number of these isolates have been identified on the series of differential varieties developed by Dr. Black, and also on those developed in the United States by Drs. Reddick, Mills, and Peterson. A close correlation between the 2 sets of differentials was soon apparent (Table 1). The 3 genes for resistance found in the United States had their counterparts in the system of Dr. Black, who had also described an additional fourth gene.

TABLE 1.—*Races of P. infestans isolated in Mexico*

Black	U. S.	International
A	A	0
B_1	D	1
B_2	BD	1,4
C	BC	2,4
D	B	4
E	—	1,3
F	—	1,3,4
G	CD	1,2
H	C	2
I	—	3,4
J	—	3
—	BCD	1,2,4
—	—	2,3,4
—	—	1,2,3,4
—	—	1,2,3,4,5 (?)

Some of the more highly specialized isolates blighted all the differentials used in both systems. These cultures were isolated originally from lesions on wild clones of *S. demissum* and also from blighting seedling lines under observation in field plots. These highly specialized isolates have been separated into 2 groups, based on their ability to attack certain clones of *S. demissum*. One group infects all the differentials, but does not infect *S. demissum* clone S-74. This might be the basis for some hope of attaining immunity, were it not for the fact that the second group of isolates[6] is able to attack not only all the differentials but also the *S. demissum* clone S-74. This clone apparently possesses a fifth gene for resistance, previously unreported. To date no clone of *S. demissum* has been found to be resistant to these most highly specialized isolates.

Our experience with other Mexican tuber-bearing species of *Solanum* has shown that nearly all of them will blight rather freely, but several levels of resistance are apparent. One clone of *Solanum bulbocastanum* Dun. to date has remained blight free. Unfortunately we have not been able to cross this clone of a diploid species with other varieties and species available. The triploid form of *Solanum cardiophyllum* Lindl. has been highly resistant. A culture of *P. infestans* was isolated from this species with great difficulty. This isolate incited small necrotic lesions, which were the only symptoms observed in the field. But here again we have failed in all our efforts to make crosses with this triploid form.

The prospects of obtaining a blight-resistant commercial potato might appear rather remote. As data and observations accumulate, it appears unlikely that blight immunity exists in the tuber-bearing species of *Solanum*. If this be the case, then the alternative is to look for the highest possible level of resistance and try to incorporate this resistance into a commercial potato. Several species, including some clones of *S. demissum*, show a high resistance to *P. infestans* even though foliage lesions are produced on them. These resistant-type lesions are slow spreading, often turn necrotic, and sporulation is sparse. Petiole and stem lesions tend to be superficial and do not kill the distal portions of the affected leaf or stem. Relatively few lesions develop on a plant, and these are more often on the lower, older leaves. This type of resistance we call "partial resistance." Some have called it "field resistance" or "tolerance." A high level of "partial resistance" is due to major genes, aided by a number of the so-called minor genes which condition the response of a given plant to the races to which it is susceptible.

Sometimes a variety such as Sebago may have a combination of minor genes to show a slight degree of resistance to all races, even though in another sense it is susceptible to all races, including the common field race "O". A somewhat higher level of partial

[6] In Table 1 this group of isolates is identified provisionally as race 1, 2, 3, 4, 5 (International System).

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resistance is demonstrated by the Dutch varieties Alpha, Gineke, and Voran, and the German variety Ostbote. All such varieties can be killed by heavy inoculations with the common field race "O". In the field, however, they show a degree of resistance that is exhibited equally toward all races of the pathogen. These "field-resistant" varieties remain green longer than varieties without this partial resistance. The practical value of this resistance lies in the fact that these varieties can be sprayed less thoroughly and less often and still be adequately protected against blight. It is the partial resistance of these Dutch varieties that we are exploiting in our commercial potato program in Mexico. We are able to control blight very effectively on these varieties, whereas more susceptible varieties are extremely difficult to maintain free from blight.

An important objective of our potato-improvement program is to increase this partial resistance in a commercially acceptable potato, so that fewer sprays are needed for blight control. We have species, such as *S. demissum* and *S. iopetalum* (Bitt.) Hawkes, that show a very high level of partial resistance; if this partial resistance could be maintained in a more commercial type potato, blight-control sprays might be unnecessary.

A second kind of resistance, which might be called "racial resistance," is due solely to the presence of 1 or more of the major genes for resistance. Dr. Black has described 4 of these genes, and we have demonstrated that a fifth gene probably exists and perhaps there are more. When these genes are present singly or in combination, they confer resistance to certain specific races. However, field experience in Mexico has shown repeatedly that racial resistance alone is not of much value. The history of many "resistant" potato varieties released in other countries supports our doubts as to the value of strictly "racial resistance"; once the varieties become fairly well distributed, they begin to blight. We believe that resistance based solely on single major genes, or on combinations of single major genes, is of very tempoary value in blight control and does not offer as much promise of permanent, practical benefit as does the partial resistance.

This is not to imply that the major genes are not of great importance, for they are. It is possible that the high level of partial resistance shown by certain clones of the hexaploid species *S. demissum* is due to the functioning of 2 or more major genes as multiples. A seedling from one of Dr. Reddick's crosses, DFR-4, is a duplex "DD" or "R_1R_1" plant. It will tolerate very severe late-blight exposure in the field and still produce a crop. While its blight resistance is not all that can be desired, it suggests that further improvement can be obtained by incorporating still other major genes, if possible also in the duplex condition. In this way perhaps we can approximate the high level of "partial resistance" to all races shown by *S. demissum*.

To find seedlings with this resistance, whether due to minor genes or major genes or a combination of both, selections should be made in the field, if possible where the most highly specialized races are prevalent. Thus one can eliminate all plants with simple major gene resistance, and select for further breeding work only those that meet higher standards for blight tolerance in the field. In our own program we are making these trials in the Toluca Valley. Should other workers be interested in testing their material here, we shall be glad to cooperate in every way possible.

The potato is a staple food for several of the earth's important populations. It could be even more widely grown and more intensively cultivated if a blight-resistant, commercial variety could be produced. The more that is known about the nature and limits of the resistance in the wild species used in breeding programs, the more likely we are to be successful. The attainment of this goal is still an exciting possibility that invites all to a maximum of cooperative effort. Only in this way shall we solve the international problem of late blight, and only in this way can we make progress in the solution of the grave problem of producing adequate food for all.

ROCKEFELLER FOUNDATION AGRICULTURAL PROGRAM
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THE USE OF WILD LYCOPERSICON SPECIES FOR TOMATO DISEASE CONTROL[1]

S. P. Doolittle

The tomato (*Lycopersicon esculentum* Mill.) is an important vegetable throughout the Americas. In the United States it is the second most important vegetable crop for the fresh market and the most important one for processing. Control of diseases is a major problem in profitable tomato production and one that is still far from a satisfactory solution.

A number of the fungi causing defoliating leaf diseases and rots of the fruit can be controlled fairly well by application of fungicides, but that is costly and laborious and often none too effective in seasons when the weather favors rapid development and spread of the pathogens. Growing resistant varieties has greatly reduced losses from fusarium wilt (*Fusarium oxysporum* f. *lycopersici* (Sacc.) Snyder & Hansen) and varieties highly resistant to verticillium wilt (*Verticillium albo-atrum* Reinke & Berthold) are now available. For other wilts, however, rotation remains the only practicable means of control. Virus diseases cause serious damage and as yet there are no means of greatly reducing the losses they cause. Nematodes take a heavier toll of the crop in the South than is realized by the average grower. Therefore, it is evident that there is pressing need for production of disease-resistant varieties of tomato.

This need for resistant varieties was recognized by the earlier investigators in the United States and breeders were successful in producing varieties with moderately high resistance to fusarium wilt. This highly important contribution to tomato disease control long remained a notable exception to the general experience in searching for resistance to disease-producing fungi, bacteria, and viruses in commercial tomato varieties. By 1940, hundreds of varieties of cultivated tomatoes from all parts of the world had been tested for resistance to a number of the more important tomtato diseases and the results were not encouraging.

When breeders and pathologists were faced with this difficulty, their obvious move was to seek for resistance in the wild species of *Lycopersicon*. This search was facilitated by the large collection of wild *Lycopersicon* species obtained by H. L. Blood and L. Tremelling, who spent several months in 1938 collecting on the western slope of the Andes and down the Pacific coast of South America. This expedition, part of the work of the then Division of Plant Exploration and Introduction, of the United States Department of Agriculture, supplied tomato breeders with a great part of the germ plasm sources now being used or tested in the United States.

This collection of wild species included numerous specimens of *L. pimpinellifolium* (Jusl.) Mill., *L. peruvianum* (L.) Mill., *L. peruvianum* var. *dentatum* Dun., *L. peruvianum* var. *humifusum* C. H. Mull., *L. hirsutum* Humb. & Bonpl., *L. hirsutum* f. *glabratum* C. H. Mull., and *L. glandulosum* C. H. Mull. These are all the known species, and nearly all the botanical forms, of the genus *Lycopersicon* except *L. cheesmanii* Riley, which is known only in the Galapagos Islands, a locality impossible to visit at the time the collections were made.

Evaluation of all this material for resistance to various tomato diseases is not yet complete, but it is already evident that the kinds of resistance in wild *Lycopersicon* species may have a revolutionary effect on tomato breeding. The earlier evidence of new and additional resistance was accumulated by a number of workers who generally tested a comparatively small number of lines of the wild species for resistance to a limited number of pathogens. More inclusive tests, however, were made by Alexander, Lincoln, and Wright (3) in 1942 and their work indicated that a number of accessions of the wild species possessed marked resistance to several pathogens.

Recently, the need for more extensive testing led to the formation of the National Screening Committee for Disease Resistance in the Tomato under the leadership of L. J. Alexander, of the Ohio Station, and M. M. Hoover, of the Section of Plant Introduction, of the U. S. Department of Agriculture. Through this committee a large number of previously untested accessions of wild *Lycopersicon* species now are being screened for resistance to most of the more destructive tomato pathogens by a nation-wide group of pathologists (2).

Today, the use, actual and potential, of wild species of *Lycopersicon* in breeding for resistance to various diseases of tomato is about as follows.

FUNGUS DISEASES.—*Late Blight.*—Since the disastrous outbreak of late blight (*Phytophthora infestans* (Mont.) DBy.) in 1946, this disease has been a threat to tomatoes in the central and eastern United States. It occurs in Canada and Mexico and causes losses in Central and South America. Although a very few commercial varieties have shown some tolerance to one or more races of the causal fungus, no marked resistance has been found in large-fruited varieties of cultivated tomatoes.

Walter and Conover (41), of the Florida Station, however, have reported pronounced resistance to some races of the fungus in 2 accessions of a small-fruited tomato (apparently *L. esculentum* f. *pyriforme* (Dun.) C. H. Mull.). Resistance is thought to be governed

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by a single gene affected by modifying factors.

Gallegly (17, 18), of the West Virginia Station, has found resistance to the common race of the fungus, but not to certain other races, in the red cherry tomato (*L. esculentum* var. *cerasiforme* (Dun.) A. Gray), which probably originated from a *L. esculentum* × *L. pimpinellifolium* cross, and in certain accessions of *L. pimpinellifolium*. This resistance is governed by a single dominant factor. Bonde and Murphy (12), of the Maine Station also have reported similar resistance in the red cherry tomato in which they found it to be a dominant characteristic. Comparatively little evidence of resistance has been found so far in other wild tomato species.

Verticillium wilt.—Verticillium wilt is a damaging disease of tomatoes in California, Utah, and some of the North Atlantic States. Some varieties with moderate resistance to verticillium wilt have been developed through selection from the cultivated tomato, but they have never proved popular from the standpoint of horticultural type and have been damaged under conditions very favorable to the development of verticillium wilt.

Recently, however, Cannon, of the U. S. Department of Agriculture, working in cooperation with the Utah Station, introduced 2 varieties, Loran Blood and VR Moscow, which possess near-immunity to verticillium wilt. These varieties were derived from a cross made by the late H. L. Blood between a semi-wild, small-fruited variety, received from J. W. Lesley, of the California Station, and a commercial variety, Century, followed by crosses of this hybrid with the varieties Stone and Moscow. Resistance is governed by a single dominant gene (35). Little data on resistance to verticillium wilt in other wild tomato species are available so far.

Early blight.—Early blight (*Alternaria solani* (Ell. & Martin) Jones & Grout) is among the most common diseases of tomato in the Americas. The chief losses result from leaf spotting that partially defoliates the plant and exposes the fruit to injury by the sun; but infection about the pedicel can cause the loss of green fruits, and seedlings are seriously injured by the stem girdling known as collar rot. Fungicides help to reduce losses from this disease, but the annual damage to the crop is extensive.

No marked resistance to the leaf spot phase of early blight has been found in the established varieties of the cultivated tomato and new varieties derived from crosses with wild species, chiefly, *L. pimpinellifolium* and *L. peruvianum*, when tested under a wide variety of conditions, have not generally shown pronounced resistance to leaf injury. However, the variety Manalucie, recently introduced by Walter and Kelbert (42), of the Florida Station, is reported to have considerable resistance to alternaria leaf spot. This variety is derived from a complex series of crosses in which *L. pimpinellifolium* entered the cross 4 times and *L. hirsutum* twice. In the recent cooperative screening tests (2) a high degree of resistance has been reported in one accession of *L. hirsutum* and marked resistance in other lines of *L. hirsutum*, *L. hirsutum* f. *glabratum*, *L. peruvianum*, and *L. pimpinellifolium*. It is not yet possible to judge, however, whether these sources will prove more valuable than those previously used in breeding for resistance to the foliar blight.

Andrus *et al* (6), of the U. S. Department of Agriculture, found high resistance to the collar rot phase of seedling infection in certain foreign forcing tomato varieties and *L. pimpinellifolium*. This resistance, found to be controlled by a single major gene (33), was incorporated in the variety Southland, the first with such resistance developed for use in the United States. This variety, like others with collar rot resistance, usually does not show high resistance to the foliar blight caused by *alternaria solani*.

Leaf Mold.—In the United States, leaf mold (*Cladosporium fulvum* Cke.) is the most important disease of tomatoes grown under glass during the winter and is damaging to the foliage of field-grown tomatoes in New York and the Middle and South Atlantic States. The disease also causes losses in field-grown tomatoes in Central and South America. Defoliation of plants in the greenhouse has never been satisfactorily controlled by the application of fungicides although certain organic compounds are fairly effective in the field. The older greenhouse varieties show differences in susceptibility but none has marked resistance.

In 1932 Sengbusch and Loschakowa-Hasenbusch (36), in Germany, reported that a strain of *L. Pimpinellifolium* showed no sign of infection when inoculated with the leaf mold fungus. The resistance was dominant and controlled by a single gene. At about the same time Alexander (1), at the Ohio Station, found similar resistance in an off-type plant that apparently came from a chance cross with *L. pimpinellifolium*. From this plant he derived a pink-fruited variety, Globelle, with high resistance to leaf mold. Guba (19), at the Massachusetts Station, introduced Bay State, a red-fruited variety with similar resistance derived from a strain of L. *pimpinellifolium*. Bailey (9), in Ontario, also developed a leaf-mold-resistant variety, called Vetomold.

As these varieties came into commercial use, however, races of the causal fungus to which each variety was susceptible were built up and soon became predominant where these varieties were used. Langford (27), in Ontario, identified 4 races of the fungus and reported that there were 3 genes, each governing a distinct type of resistance to leaf mold. Bailey (9) later reported 7 races that could be identified by reactions of hosts in the genus *Lycopersicon*. Resistance to some of these races was found in certain lines of *L. pimpinellifolium;* but when this resistance was introduced into commercial stocks, races to which the new varieties were susceptible soon appeared.

High resistance to all races identified so far seems to exist in *L. hirsutum* and *L. hirsutum* f. *glabratum*. Also resistance is reported in *L. peruvianum* and *L.*

peruvianum var. *dentatum* (2). Guba (20) recently introduced the variety Waltham Mold-Proof Forcing, which derives its resistance from *L. peruvianum* and is reported to be resistant to all races of the fungus to which it has been exposed so far. Manalucie, which carries genes from both *L. pimpinellifolium* and *L. hirsutum*, has shown high resistance to leaf mold in Florida (42). It is possible, therefore, that these wild species carry genes for resistance to races of the fungus other than those already identified.

However, we apparently are dealing with a readily mutable fungus, which promptly produces variants that infect strains of tomato resistant to previously dominant races. When such a mutant appears it soon predominates and excludes other races. Bailey (9) suggested that the tissues of the incompatible host act as a medium which may influence the type of mutation of the pathogen. Therefore, even if resistance to several races is incorporated in a single variety, its value for disease control may soon be lost. However, breeding for leaf mold resistance continues in the hope that greater success may be attained through the use of other resistant wild species.

Gray leaf spot.—Gray leaf spot (*Stemphylium solani* Weber) is a comparatively new disease of tomato foliage in the United States. It has caused serious losses in the South Atlantic States since about 1925 and now has become prevalent in some of the North Central States.

None of the older commercial varieties show any marked resistance to gray leaf spot. Andrus et al (7), however, showed that certain collections of *L. pimpinellifolium* are resistant. Hendrix and Frazier (21), in Hawaii, found that this resistance is dominant and controlled by a single gene. Using *L. pimpinellifolium* as a source of resistance, Frazier et al (16) in 1950, introduced 7 resistant varieties for Hawaii. Walter and Kelbert (42) reported that the variety Manalucie is resistant to gray leaf spot as well as to early blight and leaf mold.

Fusarium wilt.—Fusarium wilt is a prevalent and damaging disease in the southern and central United States and in some areas in Central and South America. The earlier resistant varieties developed by selection from the cultivated tomato have been of great value; but they are only moderately resistant to the wilt fungus and often are damaged when conditions are very favorable to the development of the disease.

In 1940 Bohn and Tucker (11) reported resistance, approaching immunity, to wilt in a strain of *L. pimpinellifolium*. This resistance is controlled by a single gene. The first commercial variety with this high resistance was developed by Porte and his associates (31, 32) from a cross between the resistant strain of *L. pimpinellifolium* and Marglobe. This variety, called Pan America, was introduced in 1941. It shows no evidence of fusarium wilt where moderately resistant varieties such as Marglobe and Rutgers are severely injured. Since the appearance of Pan America a number of other highly resistant varieties have been developed through the use of Pan America as the resistant parent.

Alexander and Tucker (4), in 1945, reported a second race of the fusarium wilt fungus that is pathogenic on these highly resistant varieties but rarely found in nature. Recent screening tests (2) have shown that there are lines of *L. peruvianum* and *L. glandulosum* that appear to have high resistance to both fungus races. Thus we have a source of resistance that possibly can be used for protection if race 2 should ever become prevalent in our tomato soils.

The production of varieties highly resistant to wilt is thus far the most outstanding benefit that we have derived from the use of wild species of *Lycopersicon* and is in itself sufficient evidence of their value in tomato breeding.

Septoria leaf spot.—Septoria leaf spot (*Septoria lycopersici* Speg.) ranks with early blight in its severity as a defoliation disease throughout the Americas. In the United States it is most prevalent in a central belt of States extending from Arkansas to Delaware.

Tests by various workers have shown that all commercial tomato varieties are very susceptible to septoria leaf spot. Andrus and Reynard (5), of the U. S. Department of Agriculture, have reported resistance in the variety Targinnie Red, which appears to have been derived from a chance cross with a wild species. High resistance has been found by various workers (5, 28, 29) in *L. hirsutum*, *L. peruvianum*, *L. peruvianum* var. *humifusum*, and *L. glandulosum*. Andrus and Reynard (5) and also Lincoln and Cummins (28), of the Indiana Station, found that resistance is governed by a single dominant factor. Resistant varieties are not immune but show very small, restricted lesions on the leaves.

Lincoln and Cummins (28) in studies of a cross between the resistant *L. peruvianum* and the commercial variety Rutgers found that, whereas resistance seemed to be governed by a single dominant factor, resistance equal to that of *L. peruvianum* did not appear in F_1 plants or in segregates in later generations. They suggested that this lessening of resistance may be due to the loss of modifying factors. They pointed out that varieties with moderate resistance to septoria leaf spot may not be of permanent value because host resistance does not prevent initial infection. Where this occurs and the fungus develops in what they termed the selective environment of the host, there may be genetic changes in the fungus that result in mutations which produce a new race capable of damaging varieties resistant to the previously dominant race.

As yet no commercial varieties of tomato resistant to septoria leaf spot have been introduced, but work along this line is continuing.

BACTERIAL DISEASES.—Comparatively little has been done in breeding for resistance to bacterial diseases and there are no commercial varieties resistant to

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them. Roque (34), at the Puerto Rico Station, found considerable resistance to bacterial wilt (*Pseudomonas solanacearum* E. F. S. M.) in a cross between a native wild type and a commercial variety, Louisiana Pink. In screening trials (2) a possible low level of resistance has been found in some accessions of *L. peruvianum*, *L. pimpinellifolium*, and *L. glandulosum*. Further work on this problem is in progress at the North Carolina Station.

Alexander, Lincoln, and Wright (3) found that a number of wild species showed considerable resistance to bacterial spot (*Xanthomonas vesicatoria* (Doidge) Dowson) and some also appeared to be resistant to bacterial canker (*Corynebacterium michiganense* (E. F. Sm.) Jensen). Little work with crosses between these wild types and commercial varieties has been reported.

VIRUS DISEASES.—Virus diseases cause greater loss in tomatoes than is generally realized and use of resistant varieties seems to be the only practicable means of control.

Tobacco mosaic.—Tobacco mosaic virus (*Marmor tabaci* Holmes) is prevalent wherever tomatoes are grown and no worthwhile resistance has been found in the older commercial varieties of tomatoes. However, there are sources of resistance in the wild species that have occupied the attention of tomato breeders for some years.

Doolittle et al. (13) have reported high resistance in some plants of *L. hirsutum* and in much of the breeding for mosaic resistance this species has been used as a resistant parent. Marked resistance has been found also in *L. peruvianum* by a number of workers. In the recent screening tests (2), resistance approaching immunity was found in segregates from 27 lines of this species. Holmes (22), of the Rockefeller Institute, has shown that plants derived from crosses of *L. esculentum* with *L. chilense* (regarded by Muller as a synonym of *L. peruvianum* var. *dentatum*) tend to escape natural infection. He also has found that *L. pimpinellifolium* plants are very tolerant to tobacco mosaic virus infection.

Frazier and Dennett (14), in Hawaii, found that in crosses involving *L. esculentum*, *L. hirsutum*, *L. peruvianum* and *L. pimpinellifolium* some lines showed a long delay in developing symptoms of mosaic. In our experience at Beltsville this has been true with the progeny of crosses of similar parentage. Seedling plants exhibit high resistance, but marked symptoms frequently appear after such plants have grown for a time in the field. The general experience has been that the highest resistance occurs in small-fruited progeny with resemblance to the wild parent. Larger fruited plants of better horticultural type have generally shown less resistance. Frazier and Dennett pointed out that there may be more than 1 gene or gene modifier, or both involved and we can not yet be certain whether there is actual resistance to infection or a slow multiplication, masking, or delayed movement of the virus in the plant.

Walter and Kelbert (42) reported that the variety Manalucie possesses what they believe to be 2 of the 3 genes necessary for resistance to tobacco mosaic virus and shows some tolerance of tobacco mosaic virus.

Spotted wilt.—Probably the outstanding accomplishment in breeding for virus resistance has been the production of tomato varieties resistant to the virus causing spotted wilt (*Lethum australiense* Holmes). Spotted wilt is destructive in Hawaii and causes some damage to tomatoes in California. In South America it appears to be much more generally damaging than in the United States.

Some accessions of *L. pimpinellifolium* are very resistant to spotted wilt virus and have been used as a source of resistance by breeders in Hawaii and California. The first resistant commercial variety, Pearl Harbor, was developed by Kikuta and his co-workers (25, 26) for use in Hawaii and has been very successful there. In 1950, Frazier et al (16) introduced 7 more varieties for Hawaiian use. These are resistant to spotted wilt, fusarium wilt, and gray leaf spot.

Smith and Gardner (38), in California, reported that in hybrids derived from crosses of resistant lines of *L. pimpinellifolium* with commercial varieties, none of the progeny showed a level of resistance equal to that of the wild parent. Evidence of simple Mendelian inheritance of resistance was not found.

Holmes (23) found that Pearl Harbor is not resistant to the strain of spotted wilt virus found in New Jersey but reported that 2 Argentine tomato varieties possess monofactorial resistance to the local virus strains. These lines, however, have been susceptible when tested in California.

Smith (37), in California, found high resistance in *L. peruvianum* and less resistance in *L. glandulosum*. These hosts may prove of additional value in breeding for resistance to spotted wilt, since a number of genes for resistance may be required to cope with local differences in the strains of this virus.

Curly top.—The curly-top virus (*Ruga verrucosans* Carsner & Bennett) frequently causes very severe losses of tomatoes in some sections west of the Continental Divide and occasionally appears in areas immediately to the east. Other strains of this virus are prevalent in some parts of South America. Plant breeders have long endeavored to develop curly-top-resistant tomato varieties, but no marked resistance has yet been incorporated in a variety of adequate commercial quality.

Some resistance exists in a variety called Red Peach and in a semi-wild variety, Ojo de Venada, but no very high level of resistance has been secured through breeding with them. Blood (10), who originally worked with these varieties in cooperation with the Utah Station, found a higher degree of resistance in *L. peruvianum* var. *dentatum*, *L. peruvianum* var. *humifusum*, and *L. glandulosum*. Involved crosses between these species and the cultivated tomato

gave a higher degree of resistance than had previously been developed. Since Blood's death in 1948, this work has been continued by O. S. Cannon, of the U. S. Department of Agriculture, who has secured resistance approaching that of the wild species in fairly large-fruited selections.

Virgin (40), at the Idaho Station, in 1939 found plants of *L. chilense* that were resistant to the curly-top virus and made crosses between this wild species and the cultivated tomato. This work, which was continued by KenKnight and Watson (24) and later by Watson (43) alone, has yielded selections showing considerable resistance to curly top.

Despite the difficulties involved, it seems probable that eventually we shall have commercial varieties that will be definitely less damaged by curly top than any now available.

ROOT KNOT.—None of the commercial varieties of tomato have any marked resistance to root knot nematodes (*Meloidogyne* spp.), but resistance exists in some wild tomato species. In 1941, Bailey (8), of the Tennessee Station, reported resistance to root knot nematode in *L. peruvianum* and this has been confirmed by a number of other workers.

Taylor and Chitwood (39), of the U. S. Department of Agriculture, found that *L. peruvianum* is resistant to a number of prevalent species of root knot nematodes. In recent screening trials with a number of species of *Meloidogyne* (2), it was found that a high order of resistance existed in *L. hirsutum*, *L. hirsutum* f. *glabratum*, *L. peruvianum*, and *L. glandulosum*, but individual accessions varied in their resistance to various nematode species and there was considerable variation in the reaction to these species within the various accessions.

In 1946 McFarlane et al (30) secured in Hawaii crosses between *L. peruvianum* and a commercial tomato variety by using an embryo-culture technique. The progeny were superior to the commercial parent in root knot resistance. They believed that probably resistance was dominant. Crosses with their most resistant lines of *L. peruvianum*, however, were not obtained because of cross-sterility of this species with *L. esculentum*.

In 1947 Watts (44), in Arkansas, also reported successful crosses between *L. peruvianum* and *L. esculentum* and found that the progeny showed a fair degree of resistance. He considered that resistance might be controlled by 2 dominant factors.

In 1949 Frazier and Dennett (15), in Hawaii, reported further breeding for root-knot resistance in which they used 2 lines heterozygous for resistance. These were obtained from Watts and represented material originating from a cross of *L. peruvianum* and *L. esculentum* made by P. G. Smith, of the California Station. In this work they derived a line essentially homozygous for a type of resistance that appeared to consist of resistance to root-gall formation rather than to penetration of the roots by the larvae of the parasite.

No varieties highly resistant to root knot have yet been introduced, but it appears that we shall eventually have acceptable commerical varieties with this resistance.

CONCLUSION.—When we consider what has so far been accomplished by utilizing the wild *Lycopersicon* species in breeding for disease resistance, we can be encouraged by the results. We realize that the reservoir of resistance known to exist in the wild species has been barely tapped. Progress is hampered by 1) the frequent difficulty in securing desired crosses; 2) lethal factors that make it difficult to secure desired combinations of characters; 3) the fact that as fruit size increases in the hybrid progeny the resistance to some diseases decreases; and 4) the constant problem of mutable pathogens. These difficulties emphasize the need for a continuous tomato breeding program, and they are not too discouraging.

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POTATO VIRUSES IN THE AMERICAS[1]

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SUMMARY

The American potato viruses do not form a natural group which permit us to consider them apart from those occurring in other continents. The region of the Andes, the mother country of the potato, may be considered the center of dissemination of the most common potato virus diseases, many of which have become common throughout the entire world. So we find in the region of the Andes, potato viruses X and Y, in addition to many other viruses, a few of which may not yet have penetrated into other regions. In the North American region the potato viruses of the X group have been prevalent for a long time, as have those of the Y group in the Atlantic region which, in this regard, is more closely related to Europe (40).

But if the potato viruses in the Americas do not form a natural group, they present more difficulties, owing to their diversity, and it is more important that we all work together to become better acquainted with them and to get rid of them, in our common effort to produce seed potatoes of a high standard and value.

The title of this paper could imply that potato viruses in the Americas, by virtue of some common characteristics, form an entity distinct from potato viruses in other parts of the world. In this report of research on potato viruses in the Americas, we shall try to find out if this concept is in agreement with the facts and what minor differences may appear within the group of American potato viruses.

DISTRIBUTION OF POTATO CULTURE.—The occurrence of the potato viruses is closely related to the range of potato culture. Based upon elements furnished by history and by taxonomy, the present area of potato cultivation in the Americas can be divided into 3 regions. The first region would include the table lands and slopes of the Andes, where potatoes have been grown without interruption since long before the arrival of the Spanish conquerors (54, 74, 76). Native varieties, in some way related to century-old potato varieties, are at present grown in Bolivia, Peru, Ecuador, Colombia, large sections of Chile, and in some mountainous regions of Venezuela (30). Central America and Mexico were later, possibly under Spanish influence, included in this Andean region. In the center of this region the importation of potatoes from other parts of the world has never been a problem. In the second, or North American region, consisting of Canada and the United States, the potato was first introduced 250 years ago (54, 68, 76). During the last century, private breeders in the United States succeeded in obtaining many American varieties, partly with the help of a small quantity of South and Central American potatoes, which G. E. Goodrich obtained in 1851 from Panama. In any case, in the North American region, potato culture has been firmly established for more than 100 years (13, 67, 68). In the third region, the Atlantic area of South America, potato culture has acquired great economic importance only in this century (3, 8, 55). Argentina, Uruguay, Paraguay, and Brazil belong to this third and, historically speaking, newer potato-growing area. This region depends largely on the periodical or even annual importation of seed potatoes. A large number of European potato varieties are grown in Brazil, purchased directly from the Netherlands and Germany (24, 50, 60). Argentina has made more use of American varieties imported from the United States or from Canada (8, 36, 73).

Having outlined the 3 principal regions which form the present area of potato cultivation in the Americas, recent developments in the field of potato-virus-research in these regions will be presented. A review of the literature is necessary to become acquainted with the development of potato virus research within the 3 regions of the Americas during the last 20 years.

DEVELOPMENT OF POTATO VIRUS RESEARCH.—In 1935 the Atlantic region of South America had just become virus-conscious. In Argentina a very severe degeneration of the best known potato varieties had produced devastating effects (8). The varieties Blanca and Chaqueña, according to R. Milan (36), identical with the American varieties White Rose and Spaulding Rose, introduced some 15 years before into Argentina and there extensively multiplied for many generations, were discarded in 1936. This spectacular degeneration of 2 potato varieties, which for many years had furnished valuable seed potatoes not only for the needs of Argentina but also for Brazil, Uruguay, and probably other countries as well, had marked economic and scientific consequences. Argentina imported enormous quantities of certified seed potatoes, principally Katahdin, from other countries and reorganized its system of seed potato production, stressing the importance of early elimination of virus-diseased plants (19, 37, 73). Brazil prohibited importation of seed-potatoes from Argentina, concentrated on the acquisition of certified seed from Holland and Germany, and surveyed possibilities of production of its own seed potatoes (48). The lack of seed potatoes from Argentina was an acute problem when my work on potato vi-

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ruses was initiated in São Paulo.

At that time, at least in the potato-producing areas of the State of São Paulo, potato growers usually imported certified potato-seed from Holland or from Germany, multiplied it for 3 generations, and replaced it finally with seed-potatoes purchased again from Europe. The Agronomical Institute in Campinas had already collected and published a great deal of valuable observations relating to potato production, aimed at the selection of varieties which would give the highest tuber production under the climatical conditions of São Paulo. The Dutch varieties Eigenheimer and Eersteling and the German variety Konsuragis, which in these comparative experiments had given the most satisfactory results, are still in great demand in the potato producing areas of São Paulo.

When I joined the Biological Institute in São Paulo in 1935, experiments were designed specifically to furnish data on the viruses disseminated through the potato plantations and on conditions favoring virus infections. With virus-free potato tubers of the variety Eigenheimer[2], we performed field experiments to find out which potato-regions in the State of São Paulo would be best for seed-potato production. Following the technique described by Quanjer and Gäumann (49) and others, the healthy potato-tubers were numbered and cut into halves. The 2 equal sets of potato-halves were planted, respectively, in the tableland near the City of São Paulo at an altitude of about 700 m. and in the mountains at an altitude of 1200 m. The fields were observed monthly and observations were accompanied by tests for virus identification. In these tests, in the greenhouses of the Biological Institute in São Paulo, we used the method of sap inoculation in healthy greenhouse-grown indicator plants. Before then practically all work on virus infection of potato plants in Brazil and probably in the whole Atlantic and Andean region had been restricted exclusively to field observations (61).

In these experiments we followed the increasing virus-infection of the 2 plots of potato plants grown from originally healthy stock, through 7 generations, and found a much quicker spread of virus diseases in the regions of lower altitude than in the mountains. Most of the virus isolates which we could identify by sap inoculations belonged to the potato virus Y group (59). The practical purpose of these experiments was to find a region suitable for seed potato production. When the importation of seed potatoes from Europe was entirely interrupted during the Second World War the same mountainous region in which we had found a slow spread of virus infection was chosen as an area for seed-potato production. The seed was produced by potato growers congregated in a Cooperative and the certifications were issued by officers of the Biological Institute in São Paulo (60). To ascertain the absence of virus infection in the certified stock we again used the method of virus identification by sap inoculation, as well as the tuber-index method. A greater part of the virus isolates identified in this later period of our survey was found to belong to the potato virus Y group isolated also rather frequently from tobacco fields (34, 16). Potato virus X and leafroll were also found (57, 58).

The region of the Andes likewise became potato-virus-conscious about 20 years ago. On the Pacific side, however, interest in potato viruses was aroused not so much by the immediate need for seed potatoes, but by the growing knowledge of the enormous breeding value of the native and local potato species and varieties. Much of this knowledge had been obtained by taxonomists and collectors native to the potato-producing centers of South America. But the work of these national scientists, such as Cevallos Tovar (69) and Martin Cardenas (11) in Bolivia, F. L. Herrera (31), Cesar Vargas (74), and Valeriano Soukup (65) in Peru, was supplemented by the work of foreign travelers (70) and expeditions organized with the special purpose of collecting native potatoes for breeding work. Since these expeditions had been sent out by countries in which the virus diseases already were known to cause serious trouble and were undertaken partly because of the losses caused by virus diseases, they helped spread the knowledge of virus diseases in the Andean countries. The results of a Russian, a German, and an American expedition into the region of the Andes became better known at that time (4, 8, 74). Independent of the results of these expeditions, the danger of virus infections for maintenance of a good seed-stock in the Pacific area began to be better understood, since the primitive and partially disastrous manner of potato propagation was favorable to the spread of virus degeneration. E. V. Abbot (1), G. Eckert (21), and Barducci and Guerrero (5) refer to the fact that in Peru the growers often choose the smallest tubers, the "culls" (called muñi or guache) which weigh only 10 gm., for seeds, and put several of these culls in the same hole. These same authors complain that the seed potatoes consist generally of a mixture of several varieties. In Colombia in 1947, I found the practice of putting 2 tubers into the same hole to be widespread there, also (62). During the pre-war period the governments of several countries in the Pacific area had already tried to improve the quality of the seed stock by research work in experimental stations. The work was generally aimed at obtaining clean, uniform, and heavier tubers of well defined varieties. Special attention was paid to the problem of virus degeneration by the government in Chile, where under F. M. Richatt in 1937 an agency for the certification of seed potatoes was organized, which supervised production of seeds of such varieties as President, Green Mountain, Katahdin, and others, imported from the United States and Canada (51, 52). Many growers and consumers, however, preferred the old native varieties (12, 38). In most countries of the region of the Andes the potato growers paid more attention to the name of the locality in which to buy

[2] Obtained through the kindness of Professor Quanjer in Wageningen and with the help of a group of Brazilian agronomists, especially Messrs. M. Kramer and N. Nobrega.

the seeds than to the real quality of the seed-stock. In Colombia, f. i. potatoes from Une enjoy among potato-growers a very high esteem, which in general may be deserved but is not justified in each single case.

In the United States, under the stimulating influence of W. A. Orton (46), the first agencies for seed potato certification had been set up in Wisconsin and Vermont as early as 1914 (50). In 1929 a national potato breeding program was organized, one purpose of which was to breed varieties resistant to the most common potato viruses (66, 67). This campaign was especially necessary since many of the older American varieties carry potato X virus.

Among the varieties released before 1940 were Katahdin, Chippewa, Sebago and Houma, which present a field immunity from some of the more common virus diseases (13). In 1934, E. S. Schultz and others (56) showed that seedling 41956 raised among thousands of others in the U. S. Department of Agriculture is completely immune from all strains of potato virus X. In Canada, too, a service of seed potato certification within the Department of Agriculture was organized early (47) and every year since 1927 large amounts of seed potatoes have been shipped to foreign countries (53).

POSTWAR RESEARCH.—After the war, in Brazil at least, the dependency on other countries for seed potatoes did not diminish but rather increased. This return to the practice of importation of seed potatoes, principally from Holland, was caused at least in part by the low price of good Dutch seed which made the production of home-grown seed potatoes unprofitable. Nevertheless, the scientific interest in potato-virus problems in Brazil had been aroused and in the meantime experimental work on potato virus identification had spread from São Paulo to other centers, such as the Agronomical Institute at Campinas to Belo Horizonte, to Porto Alegre, and to other places. F. Guimarães (24) tried the genetic approach with the purpose of obtaining potato varieties adapted to the Brazilian climate and resistant to diseases and pests. Since the Dutch and German varieties maintained their dominant position, the potato-viruses Y, X, and leafroll, mentioned before and rather common in the European potato area (40), continued to be found frequently in potatoes grown in Brazil. A few other viruses, such as those of aucuba mosaic, calico mosaic, and spindle tuber occasionally have been alleged to be present in Brazil, but have not been studied there exhaustively (17). On the other hand, a few potato-viruses have been identified in Brazil which seem to have a more local range and which are therefore worth mentioning. A. S. Costa and J. Kiehl (15) described under the name "necrose do topo" (tip necrosis) a potato disease caused by the virus of tomato-spotted wilt, a virus very devastating in Brazil on tomato and tobacco. The potato plants infected by this virus show necrotic rings on the middle leaves and sometimes a tip-necrosis. The tubers present dark external and internal spots, sometimes in the form of rings.

A potato disease rather common since 1937 in the potato plantations in Argentina and characterized by similar symptoms has been studied by A. M. Offermann and E. R. Vitoria (44). The disease was first confused with potato early blight and then attributed to a virus complex, one component of which was believed to be potato virus X. On the basis of the observations of A. C. Delle Coste and S. Zabala (20), and F. Valiela (72) and on the similarity of symptoms of the disease called "marchitamiento" in Argentina and "necrose do topo" in Brazil, we may consider the virus of tomato spotted-wilt, sometimes acting with other viruses, as the principal causal agent of these potato diseases in the Atlantic region. The same virus is known to cause a serious disease of potatoes in Australia.

While working in São Paulo we had many opportunities for studying the virus content of potato collections from countries of the Andean region. In 1941 we received 2 collections of Peruvian potatoes from a private naturalist. We grew them under insect-proof conditions and tested the virus content of the leaf-saps by inoculation to indicator species. In these tests we found that only 4 of the 10 varieties which sprouted in São Paulo could be shown to contain a virus (41). The varieties Mamada (from Cajamarca), Huanabana, and a third unnamed variety, contained potato virus X. The variety Serrana Negra, obtained from Central Peru, harbored a very peculiar strain of potato virus Y (41, 42). The most distinctive characteristic of the disease which this strain without the presence of X virus induces in White Burley tobacco plants consists of necrotic lines along the smaller veinlets (42, 45). Similar strains, probably originating from potatoes brought from the region of the Andes, have been described by Kenneth M. Smith and R. W. C. Dennis (64). As we also found the strains among potatoes of a collection received in São Paulo[3], we believe that they may be rather typical for the Andean region. Recently, however, we succeeded in isolating a similar strain of potato virus Y from a species of *Solanum* collected in the neighborhood of São Paulo.

The results obtained in studying the Peruvian potatoes were augmented by tests performed with 25 tubers belonging to 19 varieties[4] of Bolivian potatoes, many of which (Pucaruna, Condor imilla, Yokalla) were shown to contain potato-virus X. The special necrotic strain of virus Y mentioned before was harbored by tubers of the varieties Paceña and Phureja. This last source of virus, which taxonomically belongs to the species *Solanum cardenasii*, had been collected from a site 3600 m. above sea level, not far from Cochabamba. This observation shows that virus

[3] Through the kindness of Professor M. Cardenas of Bolivia.

[4] Obtained in August 1942 from Professor Cardenas.

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diseases may be found even in potatoes collected high in the mountains.

Similar observations were made on samples of Colombian varieties brought to São Paulo in 1947. Many of these samples, which included also semi-wild tubers, had been collected during various excursions into the Colombian Andes. Our tests performed in São Paulo showed, contrary to expectations, that many commercial varieties of potatoes from Colombia were free of virus, whereas the plants sprouted from some of the semi-wild tubers collected in altitudes between 3000 and 4000 m. harbored potato virus Y (63).

In Peru, tests on the virus content of potato tubers have been performed by Consuleo Bazán de Segura (6), who found potato X virus in some of the varieties. The greatest progress in our knowledge on the distribution of virus diseases among the potato species and varieties of Andean origin resulted from trips made by J. G. Hawkes across many countries of the Pacific coast, during which he assembled a great number of wild and cultivated species and domestic varieties of potatoes from the Pacific side of the Americas (26). The tubers, incorporated in England into the Commonwealth Potato Collection, are grown there and the resulting plants are used for breeding purposes and for many assay tests of their virus content (29). The collecting expeditions of Hawkes and also those of D. Correl (14) aroused considerable practical interest in improving the quality of seed potatoes produced in some of the Pacific coast countries. In Peru, for instance, an official service of seed-potato certification was organized which is still in its beginning and which was restricted for a few years to 1 variety, "Maco" (43). In Mexico, the Rockefeller Foundation, working in close cooperation with the Mexican Department of Agriculture, started an extensive potato improvement program. This program, supervised by J. S. Niederhauser, includes comparative observations on the yield and behavior, under Mexican conditions, of numerous foreign commercial potato varieties, and also research work with genetic methods aimed at obtaining new potato varieties with both high yield and resistance to the more devastating local diseases. Many promising clones developed within this genetic research program were derived from crosses among good foreign commercial varieties and *Solanum* species native in Mexico or South America. The native Mexican potato species, in spite of possessing genes for resistance against many important potato diseases (e.g. late blight), bear tubers of poor cooking qualities and have never been used as staple food (71).

With regard to the use of native varieties, a great difference exists between the Mexican potato improvement program and the respective Colombian project, which is also a joint enterprise of the local Department of Agriculture and the Rockefeller Foundation. In Colombia, where for many centuries the tubers of the native potato varieties have formed the staple food of the population, the present program aims also at improvement of these native varieties (like Tocana and Tucarreña). This program, originally conceived by J. G. Hawkes (27) and K. M. Fernow (22), assisted by a group of Colombian colleagues including V. Alba, N. Estrada Ramos e E. de Rojas Peña, has undergone fundamental modifications since the Rockefeller Foundation took over and is now supervised by L. E. Heiderick. The special difficulties which the Colombian Potato Improvement Program has to overcome have been correctly emphasized by V. Alba (2). These arise from the fact that most of the Colombian native varieties belong to the group of *Solanum andigenum*, the qualities of which have not been so thoroughly studied as those of the group of *Solanum tuberosum*. Thus, we still know very little about the specific reaction of each of these native varieties to virus diseases and in many cases we may confuse symptoms of virus diseases with genetic characteristics of the varieties. Masking of symptoms in tropical and subtropical regions, a difficult problem even when dealing with well-known standard varieties, represents additional difficulty in the improvement of the native Colombian varieties.

Nevertheless, much interesting work has already been done in Colombia. In the course of this effort to improve quality of seed potatoes in Colombia, a few virus diseases have been described which seem to have a more regional distribution and are therefore of special interest. One of these observed in Colombia (1946), has much in common with (and is possibly identical with) "Purple top". This disease is somewhat local in its dissemination, since it has never been observed in Brazil. The purple top disease is widespread in Colombia and in Mexico and constitutes a serious problem for the potato improvement program.

Two other diseases are still awaiting further research in Colombia. One is characterized by symptoms similar to those of potato aucuba mosaic, but is believed to be, in contrast to this latter disease, non-sap transmissible. The second new disease, called "vein-yellowing" after its most striking symptoms, is very common in the southern part of Colombia and seems to have its chief range of distribution in Ecuador. The "vein-yellowing" disease has been shown by Alba (2) to be graft-transmissible.

It is beyond the purpose of this short communication to describe the modern development of potato virus research in North America, the third of the great regions. Great progress has been achieved in this region principally by applying genetic methods to the obtaining of varieties in which good market qualities are combined with a high degree of resistance to several virus diseases as well as to other diseases of the potato (18, 25, 33, 35, 68, 75). In this work (33), native potatoes from the region of the Andes and from the Southern Atlantic region were extremely important. Also in the North American region some viruses of a more limited range have been described: spindle-tuber (9), purple-top (10), curly dwarf (23), and yellow dwarf (32, 39).

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PEOPLE, PATHOGENS, AND PROGRESS IN INTERNATIONAL DISEASE CONTROL[1]

E. C. Stakman

The control of plant diseases is so important in the problem of feeding and clothing the people of the world that it would be desirable to systematize the international exchange of information regarding the diseases and their control.

Plant diseases constitute one of the greatest obstacles to efficient and stable agricultural production. Some diseases cause terrific and wide-spread losses in a short time; others may be destructive but not conspicuous. Microorganisms may destroy plant products in transit or storage or cause serious deterioration, even when not visible to the unaided eye. The control of plant diseases therefore becomes a problem of world-wide importance.

Viruses, bacteria, fungi, and nematodes that are pathogenic to plants are disseminated principally by man, insects, and the wind. There is need for closer international cooperation in obtaining information basic to the establishment of fair and effective quarantine regulations to prevent the introduction of destructive species or physiologic races of pathogens into countries or areas where they do not now exist. But quarantines are ineffective against pathogens that may be disseminated internationally by the wind or by migratory insects; accordingly, cooperative efforts should be made to devise control measures for such epidemic diseases, either by means of fungicides, eradication programmes, or development of resistant varieties.

The problem of new diseases often is international in scope, because varieties that are resistant to the pathogens, including physiologic races as individual pathogens, in one country often are completely susceptible to those in another. There should be provision for testing resistant varieties of crop plants in all countries where similar crops are grown. This should give information regarding geographic distribution of dangerous pathogens and could result in rapid exchange and use of resistant varieties.

As new disease problems often result from the production of new races, or possibly species, of pathogens by mutation, hybridization, or heterocaryosis, the testing of varieties in those areas of the world where the same crops are grown should yield preliminary evidence of the appearance of new pathogens and indicate what needs to be done to meet the new menace.

The need for international cooperation is of course obvious to virtually all plant pathologists and to many other groups. The real job is to try to devise effective machinery for satisfying the need. Plant pathologists as individuals and scientific organizations have long tried to cooperate to the limits of their abilities and facilities. The difficulty is in obtaining the facilities for travel, for conferences, for planning, and for cooperative investigations. Certain governmental agencies in various countries, notably the U. S. Department of Agriculture, have organized cooperative effort on certain types of problems, such as problems of plant quarantine and international testing of varieties. The Food and Agriculture Organization of the UN and UNESCO are helping in certain phases of international effort in plant disease control; and the Rockefeller Foundation has contributed significantly to cooperation in the western hemisphere. There is need for sound thinking and the development of concrete plans for attacking some of the most acute problems of international importance, such as rusts of cereals, late blight of potatoes, the sigatoka disease of bananas, and many others. This is one of the assignments of the Standing Committee on International Cooperation of the American Phytopathological Society.

The degree to which plant disease problems can be studied on an international scale and the degree to which they can be controlled will determine to a considerable extent the progress in feeding the rapidly increasing population of the world.

Plant Pathology and Botany
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GROWTH AND INHIBITION OF TISSUE CULTURES ON MEDIA WITH DIFFERENT CONCENTRATIONS OF ORGANIC ACIDS [1]

Albert C. Hildebrandt, A. J. Riker and Jean L. Watertor

SUMMARY

The influence was tested of a wide range of concentrations, from 0.015 to 4 per cent, of salts of 16 organic acids on growth of marigold, Paris-daisy, periwinkle, or sunflower crown gall tissue and normal tobacco callus cultures. The acids or salts tested included acetic, citric, formic, fumaric, glutaric, glycolic, lactic, malic, malonic, oxalic, propionic, pyruvic, stearic, succinic, and tartaric acids and calcium gluconate. None of the species was able to grow appreciably on media with any one of the acids as the sole source of carbon. In the presence of 2 per cent sucrose, however, many of the acids were tolerated over a wide range of concentrations. Varying degrees of inhibition from partial to complete usually resulted as the concentrations of the acids were increased. There was considerable specificity as to the acid, species, and concentration where stimulation or inhibition appeared. Acetic, formic, or propionic acids inhibited or stopped growth completely of all species even at the lowest concentration used.

Growth is perhaps the most basic of biological phenomena. Factors encouraging growth in the early stage and limiting it with maturity are all fundamental. Emphasis appears when one considers, for example, atypical and pathological growth or the physiological interactions between host and pathogen. In these complexes, basic nutrition is obviously important, including mineral salts, vitamins, hormones, and sources of carbon or nitrogen.

The utilization of nutrients has been greatly clarified by the Warburg and related techniques. The reactions observed take place in a few hours at most. Thus, for growth requiring days and weeks and for critical concentrations, other approaches seem desirable. Valuable additional tools for studies of the metabolism of diseased and healthy tissues are provided by plant tissue cultures. The chemical formula is known for every nutrient in the medium (6). No supplements are necessary during 6 weeks of incubation. Growth is measured by weighing the tissue pieces.

Tissues of crown gall origin from a number of species have been cultured *in vitro* on synthetic media for over 12 years and through many subcultures. These and related cultures have been sensitive to the type of nutrient present and to the concentration of this material. Should certain of these materials as a result of metabolism accumulate faster than they were removed, they might influence the proper balance of factors that encourage or discourage growth. The kind and concentration of organic acids seem pertinent for understanding cell division, cell enlargement or their inhibition.

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The general background for these studies has been reviewed recently by de Ropp (1), by Riker and Hildebrandt (9), and by White (13, 14). Recent related work with callus cultures has been that of Eberts (2), Nickell and Burkholder (7), and Sapp (10). The immediate background on sources of carbon for crown gall tissues was given by Hildebrandt and Riker (4, 5) and for sources of nitrogen by Riker and Gutsche (8). Some striking results have appeared among tissue cultures of normal or crown gall origin from different species. For example, the fact that one tissue grew on a particular carbohydrate or nitrogen compound was no clear indication that tissue from a different species would grow. Furthermore, the concentration of nutrient favorable for one tissue might be unfavorable for another. Common metabolites even at fairly high dilution have inhibited growth on otherwise favorable media. Such inhibition has important implications for the regulation of normal or diseased growth.

Earlier studies (4) had tested the growth of tissue cultures of crown gall and of normal origin on media containing 0.5 per cent of each, respectively, of 16 salts of organic acids with or without 2 per cent sucrose. This concentration of 0.5 per cent for the salts of the acids had been selected because this concentration had been used in bacteriological studies of the causal organism. Without sucrose the 5 species examined generally failed to grow or grew very poorly on media with 0.5 per cent of any of the organic acids. In the presence of 2 per cent sucrose, however, a number of the acid salts were tolerated. With some, including succinic, fumaric, malic and (with 2 species) tartaric acid, the growth was equal to or better than that on the sucrose controls.

Since certain species grew well with 0.5 per cent of certain acids, while others failed partially or completely to grow, a wider range of concentrations appeared important. Special attention was given to available acids in the Kreb's cycle. However, since various plant species might not follow this cycle and because

closely similar acids might form substitute combinations that would inhibit growth, various other simple acids were included.

The present paper reports the amount of growth measured as the wet weight of tissues from 5 different species in culture on different concentrations of the sodium or calcium salts of 16 organic acids, respectively. Of further interest were: 1) the growth or growth inhibition with and without sugar; 2) differences and similarities in the responses of tissues of different origins and species, and thus 3) a clarification of metabolic processes in relation to encouragement or inhibition of crown gall and other tissue growth. An abstract of certain of these results has appeared (3).

MATERIALS AND METHODS.—The stock tissue cultures came from primary or secondary crown galls on marigold, Paris-daisy, periwinkle, and sunflower, and from normal stem tissue from the hybrid tobacco (*Nicotiana glauca* × *N. langsdorffii*). They had been maintained 8 or more years *in vitro*. The marigold, Paris-daisy, and sunflower tissues were originally freed of the inciting bacteria by mechanical selection of tissue free of the bacteria (5) and the periwinkle tissue by heat treatment (12).

All experimental manipulations were carried out under aseptic conditions in a walk-in transfer chamber as described previously (4).

Concentrated stock solutions were made of the purest grade available of each organic acid and were adjusted with NaOH to pH 6.0 which was near the optimum for all species. The concentrated stocks (12.5–25 per cent by weight) of calcium gluconate and of fumaric, glutaric, oxalic, and stearic acids were sterilized in the autoclave at pH 6.0. Concentrated stocks of acetic, citric, formic, glycolic, lactic, malic, malonic, propionic, pyruvic, succinic, and tartaric acids were sterilized with Corning fritted glass filters that held back microorganisms. The acidities of these stocks were adjusted aseptically again when necessary to pH 6.0 following either type of sterilization.

The concentrated stock organic acid solutions were then added aseptically to the basal media to give final concentrations of 0.015, 0.03, 0.06, 0.125, 0.25, 0.5, 1, 2, or 4 per cent either with or without 2 per cent sucrose. The highest concentrations of oxalic acid and fumaric acid were, respectively, 1 and 2 per cent.

Each concentration tested with the individual acids was "replicated" 3 times. Four seed tissue pieces each weighing 20 mg. were placed in each experimental flask, respectively, so that with 3 "replicate" flasks there were 12 pieces used at each concentration. The cultures were incubated in the dark at 26° C. Each experiment then was repeated 3 to 5 different times. The relative humidity in the incubation room was adjusted to about 55 per cent to minimize desiccation of the culture media.

Growth was measured by removing each tissue piece, wiping away any adhering agar or water, and weighing it on a torsion balance. The final acidities of the media after the 6-week incubation period were determined with the glass electrode. The data presented in the figures came from weighing over 50,000 cultures. The variability of the cultures has been considered and the statistical significance discussed in connection with earlier studies (5).

RESULTS.—With no other source of carbon, the marigold, Paris-daisy, periwinkle, sunflower, and tobacco tissues grew poorly or not at all on media with each, respectively, of the 16 organic acids. There were, however, distinct differences among species in their ability to tolerate or to grow slightly on media with different compounds and over different ranges of concentrations of individual acids. Marigold and tobacco tissue cultures failed to increase in weight on media with any concentration of any of the organic acids. Paris-daisy cultures increased in weight only a few mg. on media with calcium gluconate from 0.06 to 1 per cent, malonic acid at 0.06 per cent, pyruvic acid at 0.03 per cent, stearic acid at 0.125 to 4 per cent, and succinic acid at 0.5 per cent. Periwinkle tissue tolerated more of the compounds than the other 4 species. This species increased slightly in weight on media with all concentrations of pyruvic and stearic acids, calcium gluconate, and succinic acid at concentrations from 0.015 to 2 per cent, tartaric acid from 0.015 to 1 per cent, fumaric from 0.03 to 0.125 per cent, citric, formic and lactic from 0.015 to 0.06 per cent, malonic at 0.06 per cent and oxalic at 0.015 per cent. Sunflower cultures grew slightly on media with 0.125 and 0.25 per cent calcium gluconate, 0.06 per cent citric acid, 0.03 per cent glutaric acid, 0.03 to 0.5 per cent pyruvic, 2 per cent stearic acid or 0.06 and 0.25 per cent tartaric acid. The details are omitted because of their volume.

The study of organic acid tolerance and utilization at higher concentrations presented certain technical problems. For example, all the free acids in concentrated solutions had pH values of pH 1 to 3. Sodium hydroxide was used to adjust the organic acid stocks to pH 6.0. The question appeared whether this salt would inhibit growth. Therefore, tests were made with sodium hydroxide adjusted to pH 6.0 with hydrochloric acid corresponding to that added in adjusting the organic acids. These tests showed that the cultures grew well on media with a concentration of the HCl-NaOH solution that was similar to the amount of NaOH added when the organic acids were tested up to and including 0.25 per cent. At concentrations corresponding to the 0.5 per cent levels, marigold, sunflower, and tobacco cultures were inhibited 25 to 50 per cent. With an amount of salt that corresponded roughly to the 1–4 per cent organic acid media, growth was reduced or stopped. Although these conditions were not exactly comparable to those where the organic acids were neutralized with NaOH, the tests indicated that the cultures tolerated large concentrations of sodium chloride. But with the highest concentrations of 1–4 per cent, the NaOH added might have been a factor in the retarded growth. At higher

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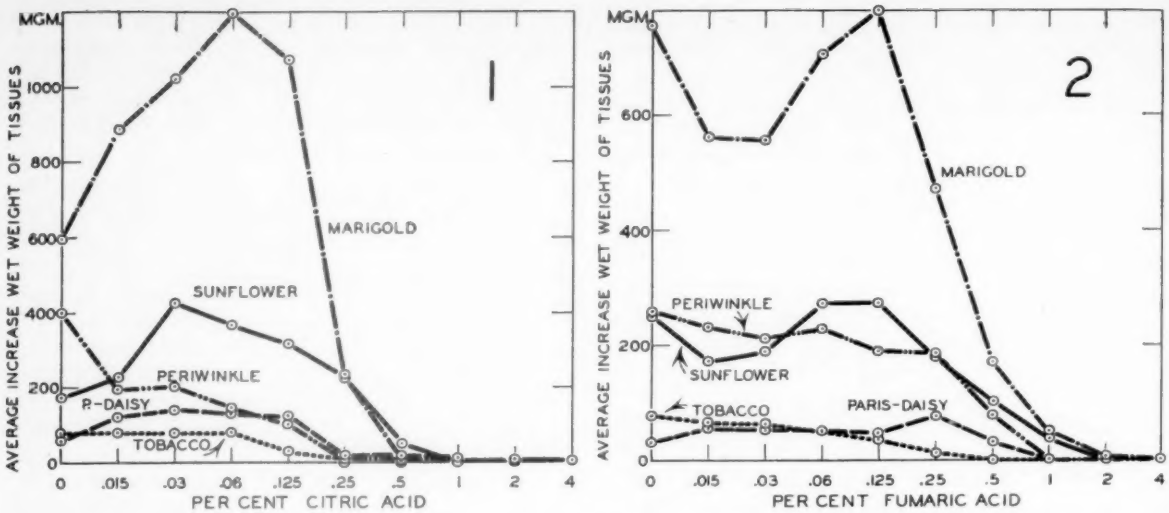

FIG. 1–2. Average increase in wet weight of marigold, Paris-daisy, periwinkle, sunflower, and tobacco tissue incubated on media containing different concentrations, respectively, of citric or fumaric acids. Each point on the curves represents the average increase in wet weight of from 36–60 tissue pieces from each of 3–5 experiments made at different times. Incubation was for 6 weeks at 26°C.

dilutions, the NaCl often stimulated growth. The role of NaCl in the osmotic values of the medium was also considered by White (11) with root tip cultures.

In the presence of 2 per cent sucrose, marigold, Paris-daisy, periwinkle, sunflower, and tobacco tissue cultures tolerated a wide range of concentrations of many of the salts of organic acids. The average increases in wet weights of the 5 species during the 6-week incubation period are summarized in Figs. 1–14. Each point on the curves represents the average increase in weight of 36–60 tissue pieces from 3–5 experiments made at different times.

These data on the tissues from 5 species have indicated various important items, including in most cases the amounts that stopped growth, those that were more or less inhibiting, and in some cases those that were mildly stimulating. Also they indicated the relative rates of growth by the different kinds of tissue and how these tissues, respectively, were different or were similar in their responses to various acids. Descriptions of these numerous points are omitted because of their length.

Graphs were not prepared for formic and propionic acids because increased growth was either lacking completely or was only slight.

Formic acid was tolerated by sunflower at 0.125, 0.5 and 1 per cent, marigold and periwinkle cultures only at 0.015 and 0.03 per cent, tobacco at 0.015 per cent, and Paris-daisy failed to grow with any concentration used.

Propionic acid prevented growth of all 5 species at all concentrations.

A comparison of the growth of the tissue cultures of each of the 5 species on the various media showed some interesting relations. Graphs with the data rearranged this way are omitted because of their length. They are on file with the American Documentation Institute.

Marigold cultures, for example, grew rapidly during the 6-week incubation period and provided a considerable range in wet weights on media with various compounds in the presence of sucrose. Marigold crown gall tissue tolerated more of the compounds over a wider range than any of the other species. Cultures on certain low concentrations of calcium gluconate, or citric, lactic, malic, malonic, oxalic, pyruvic, stearic, or succinic acids showed considerably increased weight. Most of the organic acids provided some increase in growth of the cultures over controls with sucrose alone, but growth was progressively inhibited as the concentration increased.

Paris-daisy cultures grew slowly during the 6-week incubation period. The best growth was only about 1/5 that of the marigold cultures. This species was less tolerant of the organic acids than marigold. Growth was stimulated by low concentrations of calcium gluconate, or citric, fumaric, malic, or oxalic acids. Progressive inhibition appeared with increases in concentrations of malonic, pyruvic, and lactic acids.

Periwinkle cultures, unlike Paris-daisy, marigold, and sunflower cultures, generally showed no stimulation from the various concentrations of the organic acids, except for oxalic, tartaric, or pyruvic acids. On the other hand, periwinkle tolerated a wider range of concentrations of compounds than did other species.

Sunflower tissue responded to the organic acids in many ways similar to marigold. Low concentrations of many of the acids produced considerable stimulation of growth.

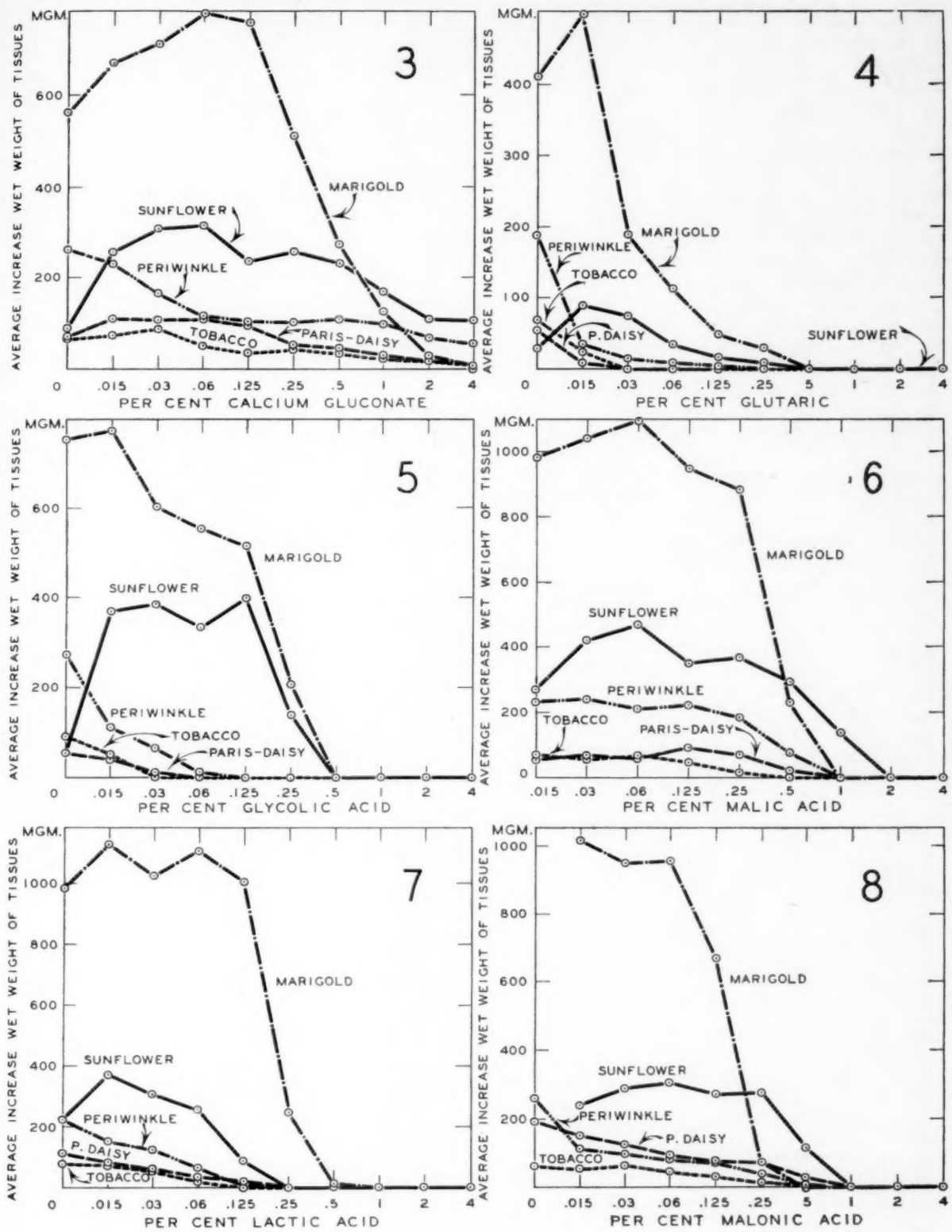

FIG. 3–8. Same as Fig. 1–2 except for organic acids as indicated.

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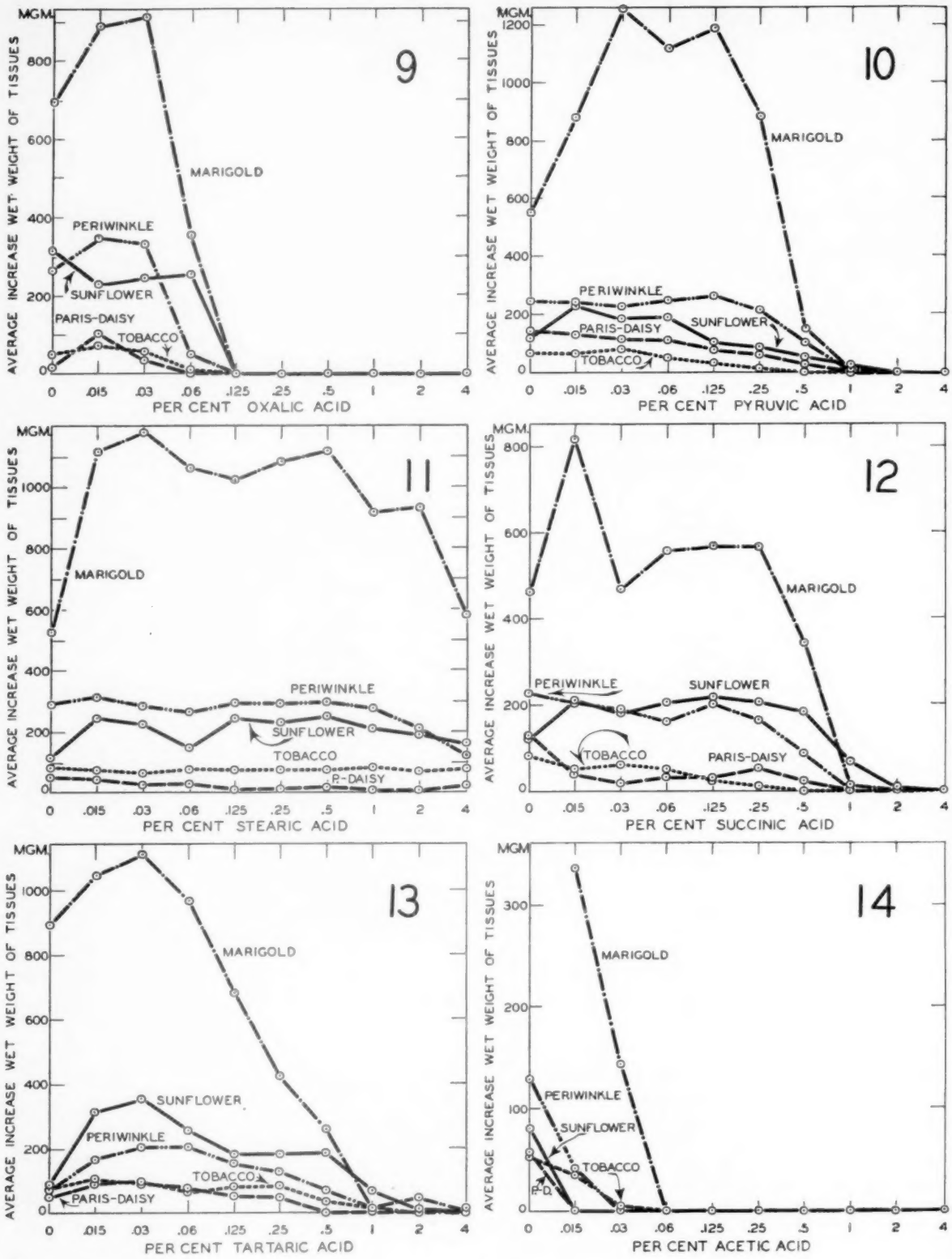

FIG. 9–14. Same as Fig. 1–8 except for organic acids as indicated.

Response of the tobacco tissue to the acids was like that of periwinkle in that many of the compounds were tolerated even though they often reduced growth at low concentrations. Only tartaric and oxalic acids stimulated growth. Calcium gluconate and citric and stearic acids were tolerated at all concentrations tested. All compounds except stearic acid progressively inhibited growth with increased concentration.

DISCUSSION.—Since growth is complex, one might expect the tissues from several plants to show striking differences in response to various organic acids, and even to changes in concentration. These data show which acids and which concentrations encourage, inhibit, or stop growth of certain specified tissues. The tissue responses frequently vary from one species to another. Thus, the physiological pictures of different tissues are more clearly defined. The question appears whether different tissues may have important variations in the organic acid metabolism.

Although the basic implications may be broad, certain reservations seem obvious. For example, except for the tobacco tissue, these callus tissues had their origins in crown gall, so they might have different responses from ordinary healthy tissue. Certainly these tissues, which are approaching the limit in simplicity for seed plants, have no benefit of substances contributed from green leaves, roots, and so on.

At the higher concentrations, the salt content may begin to interfere. The experiments with sodium chloride showed inhibition with amounts equivalent to that used at 0.5–1 per cent levels, depending on the tissue.

Results with the acids of the Kreb's cycle were of special interest. Citric, pyruvic, and malic acids considerably improved growth. Fumaric acid, however, had little or no effect. Good growth developed with calcium gluconate or glycolic or tartaric acids. However, formic, acetic, and propionic acids at relatively high dilutions inhibited growth of all the tissues studied.

The inhibition of crown gall tissue cultures by high dilutions of many amino acids (8) might be clarified if the amino group were used by the tissue and if much of the remaining organic acid accumulated. At the same time, either amino or organic acids might substitute for a similar material and form an inadequate metabolite.

Four implications of this work are mentioned but discussion is omitted.

The inhibition of diseased growth has been apparent with these simple organic acids as it has been with most amino acids (8). The question is raised whether such inhibiting substances might be chemically combined with readily used metabolites, of which many are available, and then supplied so that during metabolism the inhibitor would be produced by the diseased cells *in situ*.

In the differentiation of tissues back of growing points, the enlarging and maturing tissues with few if any cell divisions seem to contain more of organic acids. This relationship suggests various interesting possibilities.

Pathogenicity by microorganisms might deserve examination in relation to organic acids. Many fungi and bacteria produce them in fair quantity in culture. From the data presented here, some simple acids might be lethal in certain species.

Disease resistance on the other hand, might easily appear if the plant tissues contained enough of a specific acid to inhibit the growth of the pathogen. The importance of protocatechuic acid and catechol for resistance to the onion smudge fungus is well known.

In any case, a number of organic acids, respectively, at suitable concentrations appear important for understanding various phenomena involving both normal growth and growth involving various diseases.

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LABORATORY EVALUATION OF CERTAIN NEMATICIDAL MATERIALS [1]

William A. Feder [2]

SUMMARY

A laboratory technique for assaying nematotoxicity of vapors of organic phosphate insecticides against foliar nematodes is described. The fumigant action of those insecticides is utilized to kill nematodes infecting various plant tissues. Infected tissues are exposed for standard periods at standard temperatures to the confined vapors from known concentrations of candidate nematicides. Nematotoxicity is expressed as percentage nematode mortality at each concentration of toxicant. The data thus obtained are used to construct dosage-response curves for each material tested.

Within the past few years several studies on the nematicidal value of phosphate insecticides have been reported (1, 2, 3, 4, 5, 7, 9, 11, 12). Two such materials, parathion and demeton,[3] are now being employed for control of the foliar nematode disease of chrysanthemums and for the yellow bud disease of Vanda orchids, and current work indicates some success in control of bulb and stem nematode in daffodil. These insecticides are characterized by the incorporation of an organic phosphate radical into the molecule. They are highly active, are quite volatile, and appear to affect the musculature of nematodes either directly or indirectly through the nervous system. Some of these materials have only a brief nematicidal life, whereas others appear to be absorbed by the host plant and retain their activity for longer periods. The latter apparently cause the plant protoplasm to become nematicidal and are known as "systemics."

One of the major problems confronting workers using these materials is the laboratory evaluation of their nematicidal properties. These insecticides are all expensive and difficult to handle, thus the desirability of establishing nematotoxicity without using field-scale trials. There follows a description of a technique for assaying the nematicidal properties of some of these materials.

METHODS AND MATERIALS.—*Aphelenchoides besseyi* Christie, the Vanda yellow bud nematode, and *Ditylenchus dipsaci* (Kuhn) Filipjev, the bulb and stem nematode, cannot be cultured aseptically *in vitro*, but are easily found in living tissue of infected plants. When removed from the plant tissues, they live as long in solutions of parathion, TEPP, demeton, and selenium as in distilled water, even though some of these materials are nematicidal when used as sprays or drenches on infected plants (8). Therefore, in order to study the nematotoxicity of various organic phosphate insecticides another way of bringing the toxicant into contact with the nematodes had to be devised.

Studies with the Vanda yellowbud nematode, *A. besseyi* (6) revealed the marked fumigant action of parathion against it. Over 80 per cent of the nematicidal activity exhibited by parathion could be accounted for by the vapor phase of this pesticide. A simple assay technique was readily developed from this observation.

Aphelenchoides besseyi, the Vanda yellowbud nematode in Vanda orchid buds, and *Ditylenchus dipsaci*, the bulb and stem nematode in daffodil bulbs and leaf tissues were utilized in this work. The organic phosphate insecticides tested included: parathion, EPN, malathion, demeton, schradan, Shell 1836. Technical grade material was utilized whenever possible, and where formulations were used, tests were carried out using the same lots wherever possible to minimize error due to variation in formulation. The sources of these materials and their formulations are listed in Table 1.

Details of the experimental procedure will best be understood from a complete description of a single test. For this purpose the testing of demeton will be described, and the methodology discussed is typical of the technique.

PETRI PLATE TECHNIQUE.—A whole piece of Whatman No. 5 filterpaper was placed in the bottom of each 100 ml. pyrex petri dish. One ml. of the proper dilution of an aqueous solution of the toxicant was pipetted on to the filter paper in each dish. A 1½ in.

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[3] The active ingredient in Systox emulsions (Diethoxy thiophosphoric acid ester of 2 (ethyl mercapto) ethanol) is now officially named demeton.

TABLE 1.—*Trade name or common name, chemical name, formulation, and source of insecticides tested for nematotoxicity*

Trade name or Common Name	Chemical Name	Formulation	Source
parathion	O-O diethyl O-p nitro phenyl thiophosphate	25% emulsifiable	Pittsburgh Agricultural Chemical Company
demeton	Diethoxy thiophosphoric acid ester of 2(ethyl mercapto) ethanol	32.1% emulsifiable	Plant Products Corp.
EPN	Ethyl-p-nitrophenyl benzenethio-phosphonate	77% emulsifiable	E. I. DuPont de Nemours Company, Inc.
Shell 1836	Di ethyl 1 Chlorovinyl phosphate	Technical grade	Shell Oil Company Agricultural Laboratory Modesto, California
malathion	O-O dimethyl dithio-phosphate of diethyl mercapto succinate	50.3% emulsifiable	American Cyanamid Company
schradan	Octa methylpyro-phosphoramide	Technical grade	Plant Products Corp.

watch glass containing 5 similar pieces of nematode-infected leaf or bulb tissue was placed on the filter paper in the petri dish. The dish lids were then closed, and the dishes placed for 48 hours in a room having a fairly constant temperature. Usually, 3 dishes were used for each dilution, and each watch glass contained 5 separate, but similar nematode-infected tissue pieces. Thus, there were 3 replicate dishes and 15 replicate tissue pieces for each dilution.

After 48 hours exposure to the toxicant, the tissue bits were removed to fresh water in properly labeled watch glasses or pH plates. The tissues were then cut with a razor blade into 1–2 mm fragments and each fragment was teased apart with a fine needle to insure release of all nematodes present. The dissected tissues were allowed to soak for 3 hours and were then removed from the dish. The nematodes were then counted by any one of a number of techniques, and the numbers of living, injured and dead nematodes were recorded.

All the nematodes in the dish must be counted to give accurate toxicological information. This was accomplished by the suction counting pipette, or by the use of a scribed dish and a mechanical "multicoder."

TEST TUBE TECHNIQUE.—Temperature directly affects vapor pressure thereby influencing the fumigant properties of the materials under discussion. Petri dishes cannot be handled well in a constant temperature water bath, and could not be held in an incubator or closed oven, since the toxicant diffused from the dishes into the chamber and reached an equilibrium which destroyed the gradient set up initially in the dishes.

These difficulties were overcome by using 33 ml. test tubes plugged with No. 2 rubber stoppers. These took up much less space and could be used equally well in water bath or incubator. One ml. of toxicant was placed in the bottom of the test tube. A loosely fitting, non-absorbent cotton plug was then fitted into the tube just above, but not touching, the toxicant solution. This cotton plug supported the nematode-infected tissue piece directly above the toxicant, in contact with the toxicant vapors. After the tissue piece was placed on the cotton plug, the test tube was sealed with a No. 2 rubber stopper and placed at the desired temperature for 48 hours. Ten tubes, each containing a single tissue piece, were used to test each dilution of toxicant. After 48 hours' exposure to the toxicant, the tissue bits were removed from the test tubes to properly labeled watch glasses and the nematodes soaked out and counted as described under the petri dish test.

THE DOSAGE RESPONSE CURVE.—The percentage of nematodes killed by certain organic phosphate insecticides is directly proportional to the concentration of these insecticides, when temperature and exposure time are held constant. This fact is clearly shown by Fig. 1. The curve was constructed by comparing the percentage of dead nematodes with the concentration of toxicant. All data were obtained for a 48-hour exposure at 28°C. Each insecticide has a characteristic dosage response curve for these conditions. Data are plotted on log-probit paper after Horsfall (10). From such dosage response curves, the LD 50 of parathion, Shell 1836, demeton, EPN, and malathion were found

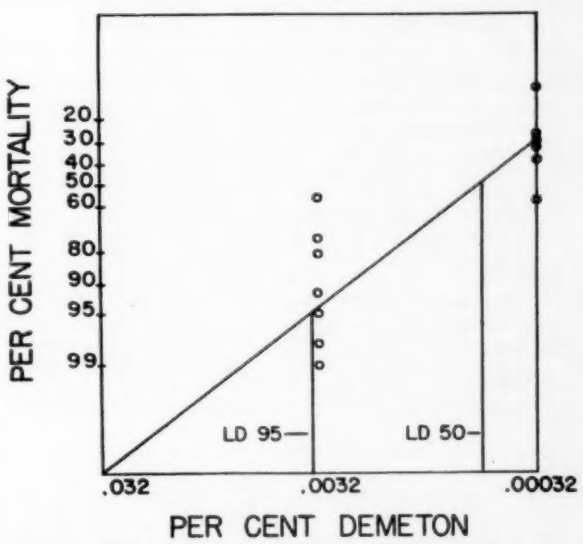

FIG. 1. Dosage response curve of demeton against *Ditylenchus dipsaci* (Kuhn) Filipjev expressed in log-probits. Tested at 28°C. for 48 hours.

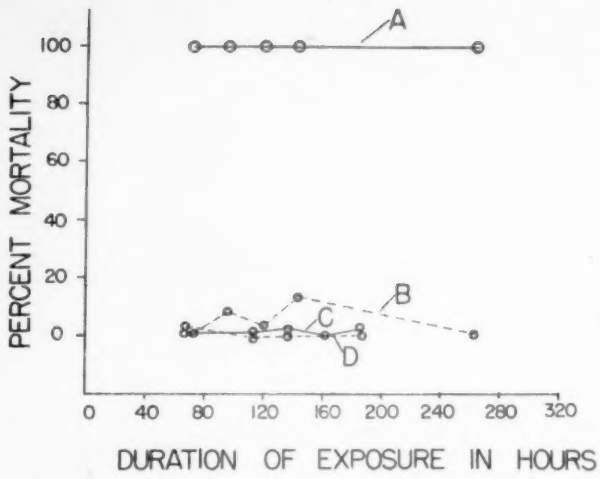

FIG. 2. The interaction of temperature and exposure time upon the nematotoxicity of demeton against *Ditylenchus dipsaci* (Kuhn) Filipjev. Curves A and C represent nematodes exposed to demeton vapors at 28°C. and 20°C. respectively, and curves B and D represent nematodes exposed to distilled water vapors at 28°C. and 20°C. respectively.

to be 1.0, 1.5, 3.0, 4.5, and 5.0 p.p.m., respectively.

The dosage response curves are a direct expression of the fumigant activity of the insecticide, which activity is in turn dependent upon the temperature and exposure period. Fig. 2 shows the change in fumigant activity of demeton brought about by varying the temperature and exposure time. To obtain these data, the test tube technique was employed using 0.032 per cent demeton and nematode-infected daffodil leaf tissue. The desired temperature was maintained by immersing the test tubes in a mercury-relay controlled, constant temperature water bath. A series of 4 tubes was removed every 24 hours and the percentage of nematode kill recorded. The tests were run 3 times and the results averaged and plotted.

At 20°C., the fumigant action of 0.032 per cent demeton was sharply curtailed. Whereas a large percentage of the nematodes were injured, 100 per cent kill was not obtained after 186 hours' exposure. In marked contrast, at 28°C., 100 per cent kill was obtained after 48 hours' exposure.

DISCUSSION.—It appears possible to construct a dosage repsonse curve for the nematotoxicity of certain organic phosphate insecticides possessing fumigant activity. From these curves the LD 50 and LD 95 values at specific temperatures are readily obtained, thereby permitting a comparison of the nematotoxicity of these insecticides.

Such weakly volatile nematotoxic materials as sodium selenate and schradan do not show nematicidal activity in this test. Materials other than organic phosphate insecticides which possess fumigant activity however could probably be screened for nematicidal action using the outlined technique.

The fumigant action of the materials depends upon their vapor pressures. Therefore, factors affecting vapor pressure, such as temperature, purity of material, and diluent, must be citically controlled. Uniform infestation of plant materials is seldom encountered. Therefore, every effort should be made to select similar material for each test, and tests should be repeated often to insure a degree of accuracy.

Although this screening technique cannot be used to predict effective dosage rates for field application, the results obtained may well be used as a guide to field scale trials. Proper use of the technique should result in the field testing of only the most promising nematicidal materials.

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THERAPY OF NEMATODE INFECTIONS OF PLANTS WITH 3-P-CHLOROPHENYL-5-METHYL RHODANINE [1]

Armen C. Tarjan

SUMMARY

Aqueous emulsions of 3-p-chlorophenyl-5-methyl rhodanine applied at the rate of 2 gm. per sq. ft. to potted tomato plants infected with *Meloidogyne incognita* resulted in complete control of these root-borne parasites. Tomato plants failed to become infected when transplanted into nematode-inoculated soil in which the chemical as a powder had been mixed at rates as low as 0.5 gm. per sq. ft. of surface. Possible modes of action of the chemical against the nematodes are discussed.

Chemical control of nematodes that parasitize numerous food and ornamental crops has been largely confined to the use of soil fumigants as pre-planting treatments for annual, biennial, and a few perennial plants. Established nematode infections of perennials have been eliminated by various heat treatments of the plants before planting and some nematode infections of growing plants have been greatly reduced or eliminated by application of some organic phosphates such as parathion (6) and systox (3) or sodium selenate (5) to the soil. The organic phosphates, however, have a high degree of toxicity to warm-blooded animals[2] while sodium selenate is a deadly poison and should never be applied to soil in which food crops might be grown.

In systematic nematicide screening tests and in additional greenhouse experiments, 3-p-chlorophenyl-5-methyl rhodanine (Compound N-244[3]) has shown promise as a contact nematicide and as an ovicide when used against egg masses of the root-knot nematode, *Meloidogyne incognita* (Kofoid & White) Chitwood. When the chemical was applied in aqueous emulsion as a soil drench at rates as low as 3 gm. of the active ingredient per sq. ft. of soil to potted, Rutgers variety tomato plants infected with the root-knot nematode, infections were greatly reduced in comparison with untreated, infected control plants (7). Roots of treated plants exhibited a very few undeveloped egg masses, indicating that development and reproduction had been greatly retarded in those nematodes which had escaped death.

In more extensive greenhouse experiments on potted, infected tomatoes, complete control of the parasites was obtained when aqueous emulsions of the chemical were applied to the soil at rates as low as 2 gm. per sq. ft. (Fig. 1). Treatments applied at the rate of 1 gm. per sq. ft. reduced the number of female nematodes infecting roots of treated plants to less than ½ the number found in roots of untreated control plants. The chemical, however, appeared to be totally ineffective when applied in the dry state at the rate of 4 gm. per sq. ft. to the upper inch of soil around crowns of infected plants. An aqueous emulsion containing 10,000 p.p.m. of the test compound and applied as a foliar spray to nematode-infected plants was likewise ineffective in reducing root populations.

Non-infected tomato plants were transplanted into 5-in. pots in which the compound as a powder had been mixed with the soil at the rates of 2, 1, and 0.5 gm. per sq. ft. of surface. Each replicate pot was then inoculated with 5 egg masses of the root-knot nematode. Examination of roots of the plants, 2 months later, showed that 100 per cent control had been achieved, at all dosages of chemical treatment of the soil, in comparison with the control plants whose roots bore an average of 943 females per plant.

It has been stated that 3-p-chlorophenyl-5-methyl rhodanine is an effective soil fumigant (4) as well as a promising chemical for treatment of rough rice and hulls for control of a nematode disease of rice plants known as "white tip" (1, 8). However, reports on the therapeutic use of this material for the effective treatment of growing, nematode-infected plants are unknown to this writer. Chemotherapy has been defined as treatment of a host with a compound so that the action of the compound occurs inside the host when the host is diseased (2). Furthermore, the compound may a) kill the pathogen as it enters the host, b) free the host of an established pathogen, or c) mitigate the disease. On the basis of the results obtained and in accordance with the above-stated definition, 3-p-chlorophenyl-5-methyl rhodanine can rightfully be regarded as a chemotherapeutant when applied in aqueous emulsion.

The chemical appears to exhibit no systemic toxicity[4] when used as a therapeutant. Root systems of treated plants, when examined approximately 4 weeks after treatment, were composed primarily of normal, healthy-appearing secondary roots, with a conspicuous absence of disfiguring, nematode root-knots. On the basis of numerous observations of roots of treated plants it is suggested: a) that the chemical may directly kill

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[2] Parathion—Acute oral LD 50 to female rats 6 mg./kg. Systox—Acute oral LD 50 to female rats 9.4 mg./kg.

[3] Supplied by the Stauffer Chemical Company. Actute oral LD 50 to male albino rats 690 mg./kg.

[4] Systemic toxicity is suggested as that condition in which the deleterious effects of a chemical are manifested by all parts of the treated plant. Local toxicity, in contrast, is the injurious effect of chemical treatment applied to a specific part of a plant with no apparent effect resulting on other parts of the same plant. The cooperation of Dr. F. L. Howard in proposing these terms is gratefully acknowledged.

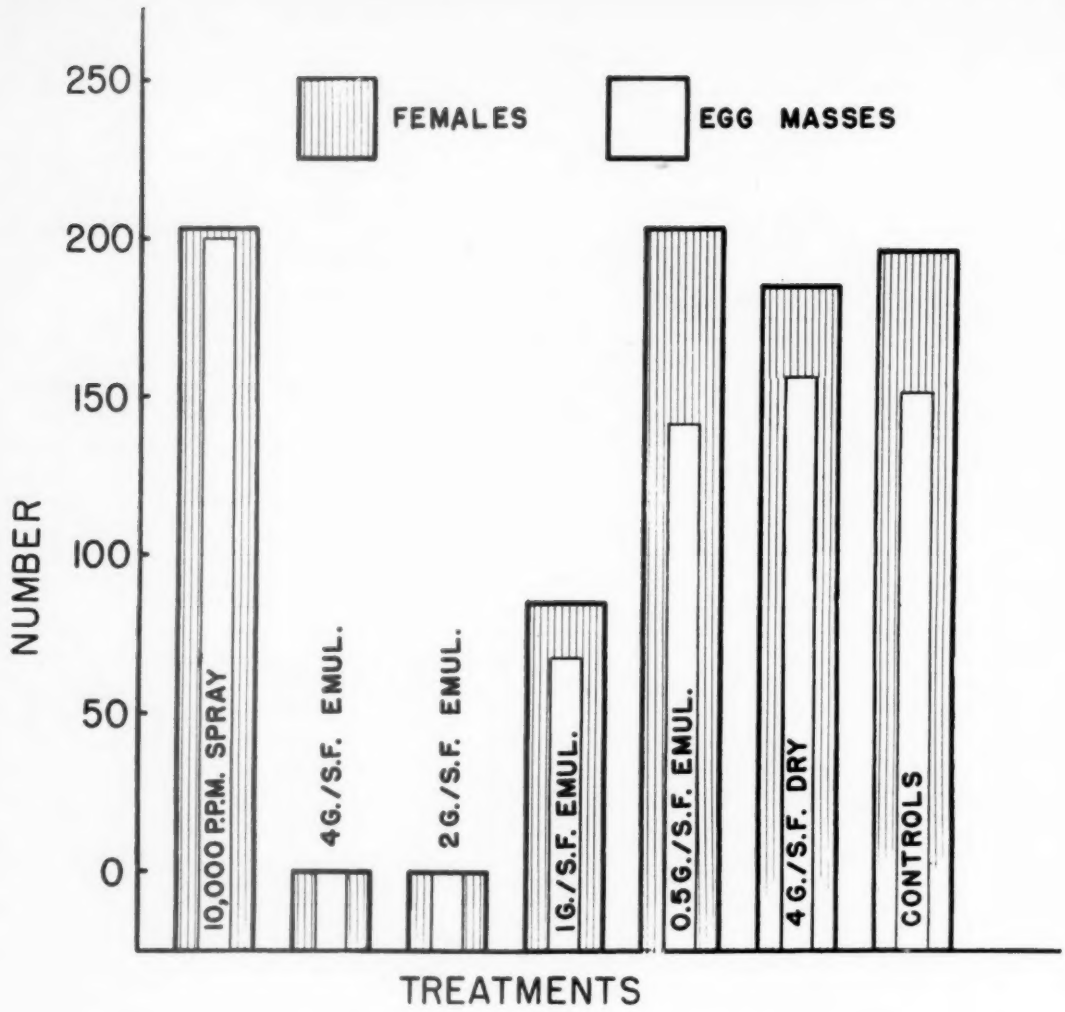

FIG. 1. Effect of chemical treatment on nematode population in roots.

the nematodes in the root tissue and the true identity of the knots formed by the eradicated pests is soon obscured by accelerated growth of adjacent tissues following treatment, b) that the chemical may kill the roots harboring the parasites which subsequently starve to death, or c) that a combination of the 2 may occur. Secondary roots then develop normally, unhindered by nematode attack.

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A PRELIMINARY INVESTIGATION OF TOXINS PRODUCED IN VITRO BY VERTICILLIUM ALBO-ATRUM [1]

Ralph J. Green, Jr. [2]

SUMMARY

Production of certain toxic substances by *Verticillium albo-atrum* R. & B. in vitro was reaffirmed. Responses induced in tomato cuttings included wilting, chlorosis, and desiccation. Wilting, as a criterion for assessing the toxicity of culture filtrates, was found unreliable. Precipitation of the toxic fractions from culture filtrates with 95 per cent ethyl alcohol destroyed the activity of the toxins; host responses encountered in the presence of resuspended precipitate were the result of mechanical occlusion rather than the activity of a toxic principle.

Selective precipitation methods and various other analyses indicated the presence of a proteinaceous and a polysaccharide fraction in the filtrate from 30-day cultures of *V. albo-atrum*. The proteinaceous material apparently was responsible for responses observed in cuttings; the polysaccharide fraction induced vascular discoloration and gummosis only in the absence of the proteinaceous fraction.

Excessive accumulation of nitrite ions in the culture solution was transitory and failed to coincide with the maximal wilt activity of the culture filtrate.

Comparison of the normal growth curve of *V. albo-atrum* and the time of elaboration of the toxic substances in vitro revealed that autolysis of the elaborated cells had begun before the presence of any toxic activity could be ascertained by the reaction of test cuttings.

Verticillium wilt, incited by *Verticillium albo-atrum* R. & B., is a serious problem in a large number of economic plants (15). Although the symptom syndrome is well known, there have been few recent investigations of the manner in which the fungus induces the typical host responses. The assumption prevails that a toxic substance, elaborated by the pathogen, is responsible, but the occurrence and nature of the suggested toxins is poorly defined.

The purpose of this investigation was to evaluate the significance of the various toxins described by earlier investigators as to their role as incitants of the wilt reaction in plants infected by *V. albo-atrum*. In addition, an attempt was made to characterize these substances more precisely as to their chemical nature and mode of action in the host plant.

LITERATURE REVIEW.—In 1922, Bewley (1) demonstrated that a substance, capable of inducing a wilt reaction in tomato cuttings, could be precipitated from the culture filtrate of 30-day cultures of *V. albo-atrum* by the addition of an equal volume of 95 per cent ethyl alcohol. The activity of the precipitate, after resuspension in distilled water, was destroyed by heating at 100°C. for 5 minutes. Picado (13) conducted similar experiments but found that heating increased rather than decreased the toxicity of the precipitate. He also described a second fraction, recovered after trituration and autolysis of the mycelial mat, which was thermostable.

Dufrenoy (3), Dufrenoy and Dufrenoy (4), and Bordas and Joessel (2) considered the wilt reaction to result from accumulation of nitrite ions in the vascular elements of the host plant. In vitro experiments demonstrated that *V. albo-atrum* exhibited strong reducing tendencies in the presence of nitrate ions.

More recently, Nelson (11) described the production or activation of a proposed toxin, elaborated in vitro, as being dependent upon strong light. Although he was unable to demonstrate a photochemical response or conditioning, he found that, generally, the test cuttings failed to wilt in an otherwise potent culture filtrate if maintained in the dark or if the flasks containing the filtrate were adequately covered with light-proof paper. He also found that heating the filtrate increased the toxicity.

It is apparent that there is little agreement concerning the nature of the toxic substance elaborated in vitro or the conditions under which the host responses are encountered. In the investigations cited above wilting was the sole criterion for assessing the toxicity of the culture filtrate. Examination of infected plants reveals that wilting is an ephemeral and unreliable symptom which may not occur at all, depending upon environmental conditions.

METHODS AND PROCEDURES.—A culture of *V. albo-atrum* highly pathogenic to tomato (*Lycopersicon esculentum* Mill.) was used.[3] The basal medium for all growth and toxicity studies was Czapek's (Dox) solution having an initial pH, after sterilization, of 4.8–5.0. Cultures were incubated at a constant temperature of 24°C. ±1° and a constant relative humidity of 85 per cent.

Unless indicated otherwise, cultures were grown for 30 days in still culture in 500 ml. Erlenmeyer flasks, each containing 100 ml. of the basal medium. The mycelial mats were then removed by filtration and the filtrate rendered cell-free by passage through a Seitz filter. The culture filtrate was amber in color and exhibited a slight odor. Final pH readings were made and the volume of the filtrate restored to that of the

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[3] The writer is indebted to Dr. Stephen Wilhelm, Division of Plant Pathology, University of California, for providing this culture.

original solution by the addition of sterile distilled water. The pH was adjusted to 6.5 and the following dilution series prepared: 1:2, 1:3, 1:5 and 1:10. The undiluted culture filtrate was not used for testing since it was apparent that a wilt reaction occurred as a result of exosmosis.

Bonny Best tomato seedlings, aproximately 8 inches tall, were utilized as test plants. The roots were excised under water and the seedlings placed 1 per flask in the various fractions of the culture filtrate. Adequate checks and controls were maintained and the tests were usually conducted under constant florescent lighting. Readings were made at regular intervals for 72 hours. The following classes were defined to evaluate the host responses: 0—no symptoms; 1—primary wilting of the leaflets and petioles; 2—complete wilting of the leaves and stem; 3—chlorosis and/or necrosis, no wilting, and 4—wilting, chlorosis and/or necrosis.

The 30-day culture filtrate was precipitated initially by the addition of an equal volume of 95 per cent alcohol. The supernatant liquid, which was clear after centrifugation, was removed and the precipitate washed, dried in vacuo over calcium chloride, and resuspended in a volume of sterile distilled water equal to that of the original culture filtrate. The supernatant liquid was recovered by vacuum distillation of the ethyl alcohol.

Various methods were employed to characterize the constituents of the culture filtrate more precisely. The proteinaceous fractions were removed by the Zn $(OH)_2$ precipitation method suggested by Somogyi (16). The precipitate was thrown down by centrifugation at 2500 r.p.m. for 30 minutes and the supernatant liquid, which retained the amber color typical of the unaltered filtrate, was collected. In addition, analyses were made to determine the nitrate, nitrite, and polysaccharide levels in the culture filtrate at various times. The nitrite concentration was determined by the method described by Rider and Mellon (14) and the nitrate levels were measured by a modification of the brucine method (12). Polysaccharide accumulations were determined by comparing the difference between the total carbohydrate levels and the reducing sugars in the cell-free filtrate. The difference was considered to represent polysaccharides. The reducing sugars were determined using the copper reagent suggested by Somogyi (17) and the chromogenic reagent described by Nelson (10). The total carbohydrate levels were determined by the method suggested by Loewus (9). A Universal electrophotometer was used for all colorimetric determinations.

Results.—The initial response of the Bonny Best tomato cuttings when placed in various dilutions of the 30-day culture filtrate was a tendency to wilt, especially in the 1:2 and 1:3 dilutions. Recovery was partial or complete within 24–72 hours, during which time there was a progressive intercostal blanching of the leaflet blades. This was followed by chlorosis and desiccation, with symptoms most intense in the lower leaves. At the dilution of 1:5 or higher, wilting was usually slight and the cuttings recovered full turgidity within a few hours. Chlorosis and desiccation proceded independently of the wilt response, however. There was no evidence of vascular discoloration after 72 hours in any test cutting.

With the toxicity of the culture filtrate established, a 1-liter sample of culture filtrate from 30-day cultures of *V. albo-atrum* was treated with ethyl alcohol in the manner described in the section on methods and procedures. The precipitate was resuspended in sterile distilled water and samples withdrawn for testing. The supernatant liquid, recovered after vacuum distillation to remove the alcohol, was prepared in a similar manner. Test cuttings of Bonny Best tomato seedlings were placed in each of 10 replications of each fraction. An additional 10 replications were prepared of the unaltered culture filtrate and distilled water for checks and controls, respectively.

The tomato cuttings in the resuspended precipitate wilted severely within 1–4 hours but failed to exhibit any other symptoms typical of the response of cuttings to the unaltered filtrate. When 5 of the test cuttings were removed to tap water and approximately 1 inch excised from the stem base, they recovered full turgidity within an hour. No further symptoms developed in these cuttings during the next 72 hours. None of the test cuttings in the supernatant liquid exhibited a response of any kind. These experiments were repeated several times with similar results.

The results seem to indicate that the toxic fraction responsible for flaccidity, chlorosis, and desiccation was readily removed from the culture filtrate by precipitation with 95 per cent ethyl alcohol. However, the method so altered the toxic substance that, when resuspended in sterile distilled water, it no longer induced the same response in tomato cuttings. The wilt reaction observed, i.e., flaccidity of leaves and stem, was apparently due to mechanical occlusion and was not an irreversible response typical of a true toxic reaction.

The $Zn(OH)_2$ precipitation method previously described is specific for proteinaceous materials. The supernatant liquid, collected after precipitation of a 1-liter sample of 30-day culture filtrate, retained the amber color typical of the unaltered filtrate. After adjusting the pH to 6.5, the supernatant was prepared in a dilution series of undiluted, 1:2, and 1:5 for testing. At the end of a 72-hour period, none of the cuttings exhibited foliar responses similar to those induced by the unaltered filtrate. An examination of the vascular cylinder, however, revealed a marked discoloration of the lower stem. The brown gum-like deposit extended to the first node, in some instances, and free-hand sections of the stem demonstrated that the discoloration was due to an accumulation of extraneous materials in the lumina of the vessels.

It was apparent that at least 2 fractions were present in culture filtrate from 30-day cultures of *V. albo-atrum*. The first of these incited flaccidity,

chlorosis, and desiccation while the second caused vascular discoloration of the lower stem. Discoloration of the vascular cylinder never occurred except in the absence of the wilt-inducing substance.

Further tests with the 30–day culture filtrate indicated that the toxic fraction responsible for chlorosis and wilting was thermolabile when heated at 100°C. for 5 minutes and was easily hydrolyzed by mineral acids. When samples of the culture filtrate were exposed to ion exchange resins, this fraction was removed in the presence of a cation resin (Amberlite IR-100H) but unaltered by the anion resin (Amberlite IR-4B). Attempts to recover the toxic fraction from the cation resin after exhaustion were unsuccessful.

To consider the role nitrites may play in the wilt syndrome, a series of 30 500-ml. Erlenmeyer flasks, each containing 100 ml. of the basal solution, were inoculated and incubated at controlled temperature and humidity. At 7–10 day intervals, 3 flasks were withdrawn at random and the culture filtrate rendered cell–free by filtration. After restoring the filtrate volume to that of the original solution (300 ml), analyses were made to determine the nitrate and nitrite levels following the procedures described above.

At the same time, analyses were made to determine whether or not polysaccharides were present in the culture filtrate. It was assumed that the amber-colored fraction apparently responsible for gummosis and discoloration of the vascular cylinder in certain test series might represent a polysaccharide of some type. Foster (5) described hydrophylic polysaccharide "gums" as common metabolic by-products of certain fungi and Thomas (18), Hodgson et. al. (7), and others have associated polysaccharides with the wilt response induced by other fungi. As described earlier, the method used to determine the polysaccharide accumulation was to compare the reducing sugar levels to the total carbohydrate accumulation. Sampling was continued over a 45-day period.

Figure 1 presents the relationship of the nitrates to nitrites in the culture solution over a 65-day period. The data exhibit some striking results. It is apparent that the reduction of nitrates proceeded rapidly in the early growth stages. The accumulation of nitrites, which exceeded 850 p.p.m. after 12 days, was transitory and, after 19 days, was not significantly different from the controls (2.4 p.p.m.). The slight rise in the nitrite levels after 50 days probably represents autolytic release of nitrogenous substances into the culture solution. There was no indication of the high nitrite levels being toxic to the fungus and this observation is supported by the data presented by Johnson (8). He found that *V. albo-atrum*, isolated from peppermint (*Mentha piperita* L.), readily utilized nitrite ions as a nitrogen source.

Figure 2 represents the polysaccharide accumulation, as determined by the difference between reducing sugars and total carbohydrates in the culture filtrate. The data indicate that there was no substantial increase in the polysaccharide levels until after 20 days growth but thereafter a steady increase in concentration occurred. To ascertain the general nature of this fraction, a 1-liter sample of 30–day culture filtrate was precipitated first with $Zn(OH)_2$ and the amber colored fraction was then precipitated with 95 per cent alcohol. After collecting and washing the precipitate, it was suspended in 25 ml. of distilled water, the pH adjusted to 1.2 with 5N HCl, and

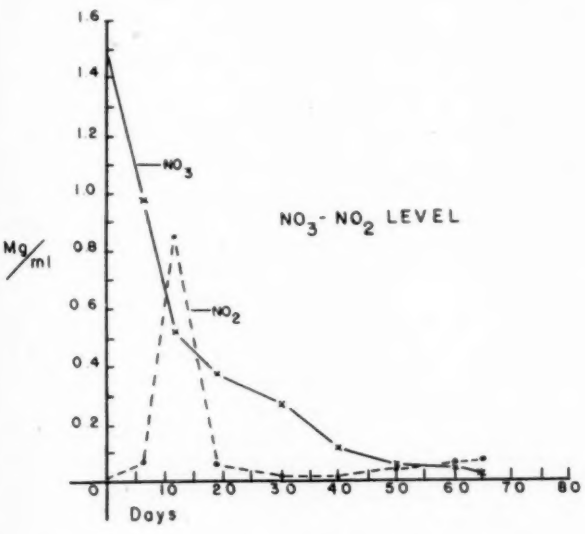

Fig. 1. A comparison of the NO_3–NO_2 levels in the culture filtrate from cultures of *V. albo-atrum* at various times after inoculation.

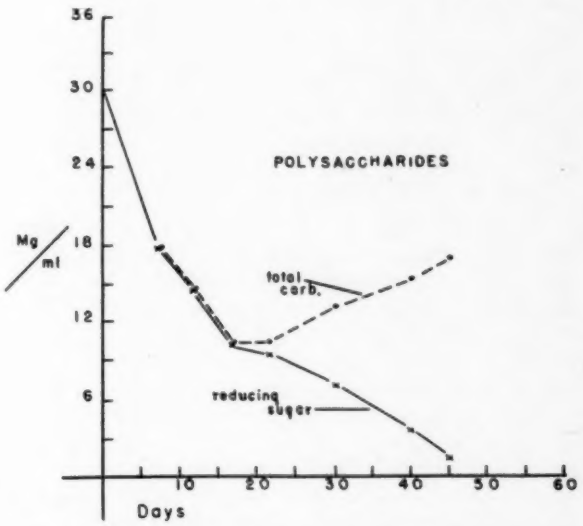

Fig. 2. Determination of the polysaccharide levels in culture filtrates from cultures of *V. albo-atrum* by comparing the reducing sugar levels to the total carbohydrate accumulation.

refluxed for 4 hours. Following the scheme proposed by Hawk, Oser, and Summerson (6), the polysaccharide fraction was identified as a polymer of hexose sugars with glucose as the principal, if not only, constituent.

Data presented thus far have given no indication of the time, in relation to the normal growth curve, that the toxic fractions are elaborated by *V. albo-atrum*. To obtain this information, 100 Erlenmeyer flasks (250 ml. capacity) containing 50 ml. of basal medium, were inoculated with 0.1 ml. of spore suspension and incubated at controlled temperature and humidity. At 5 day intervals, 6 flasks were withdrawn at random, the filtrate removed and the mycelial mats dried to a constant weight. The mean mat weight was taken for each sampling and the pH of the culture filtrate determined. After the pH was adjusted, appropriate dilutions were made and test cuttings of Bonny Best tomato seedlings were placed in samples of the filtrate collected at each sampling. In this manner, a correlation could be established between the relative age of the pathogen, in vitro, and the appearance of the toxic fractions.

Figure 3 represents the normal growth curve for *V. albo-atrum*, as determined by increases in total dry mat weight. It will be noted that maximum growth was reached about 20 days after inoculation. The rather sharp decline which followed apparently represented the onset of autolysis. The pH range during the period covered by the growth curve exhibited a shift towards neutrality and remained constant until late in the growth period.

Responses of the tomato cuttings to the culture filtrates are in Table 1. The potency of the culture filtrate, as determined by severity and rate of development of symptoms in test cuttings, was directly proportional to the age of the culture. Reference to the growth curve in Figure 3 reveals that autolysis had begun before severe symptoms were induced. It is assumed that the responses noted at the 10 and 15 day samplings, which were not repeated in the next 2 samples, reflect the maximal nitrite accumulations. From Figure 1 it can be noted that the maximum nitrite concentration (855 p.p.m.) was recorded about 12 days after inoculation.

No detailed experiments were conducted to determine what effect strong light had on the wilt potential of the culture filtrate from 30–day cultures of *V. albo-atrum*. It was observed, however, that cultures incubated in the dark and under intermittent light gave identical responses when the culture filtrate was tested. Tests were conducted both in the dark and under constant light. Admittedly, these results are not conclusive but they give evidence that the presence or absence of strong light had no apparent effect upon the wilt potential of the culture filtrates from cultures of *V. albo-atrum* utilized througout this research.

Discussion.—The results of this investigation reaffirmed the existence of toxic substances in the culture filtrate from 30–day cultures of *V. albo-atrum*,

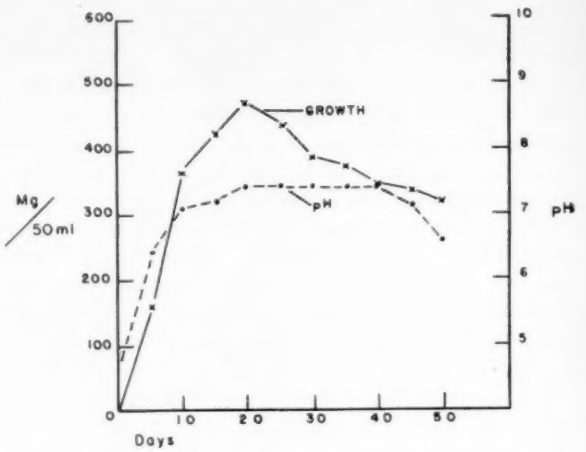

Fig. 3. Normal growth curve of *V. albo-atrum*, isolated from diseased tomato plants, and pH changes during growth in Czapek's (Dox) solution.

demonstrating that the responses of test cuttings included wilting, chlorosis, and desiccation. Wilting, i.e. flaccidity of leaves and stem, was an unreliable symptom as an index of toxicity. Earlier investigators apparently failed to recognize that alcohol precipitation of the toxic substances in the culture filtrate so altered these substances that the wilt response was the result of the mechanical occlusion of the vascular cylinder rather than the response to a toxin.

The selective precipitation methods employed indicated that the fraction responsible for the host responses other than vascular discoloration was a proteinaceous material. The evidence presented clearly indicated that this fraction was not present in detectable amounts until after autolysis had begun. Thus, the logical conclusions to be drawn are that the toxic substance is an autolytic by-product and probably peculiar to in vitro culture. In a similar manner, it seems unlikely that the host reactions observed are the result of accumulations of nitrites in the plant tissues. This suggestion was originally proposed when it was demonstrated that *V. albo-atrum* reduces nitrates readily in vitro. No consideration was given to the relationship between the maximal nitrite concentrations and the most intense responses noted in test cuttings. It has been demonstrated that the transitory accumulation of nitrites in the culture solution, though striking, failed to correspond to the period in the growth curve when the symptoms induced in test cuttings were most severe. It is proposed that the nitrite levels observed reflect metabolic activity on a substrate rich in nitrates (1.46 mg/ml) rather than a potential toxin. Cultivation of the pathogen on a less favorable nitrogen source or a substrate with nitrate levels more on the order of those observed in the sap stream of susceptible plants will probably support this suggestion.

The vascular discoloration noted when cuttings were placed in culture solutions previously treated to re-

TABLE 1.—*Response of tomato cuttings to culture filtrate of V. albo-atrum at various ages*

Time (hours)	Age (days) of culture filtrate								
	5	10	15	20	25	30	40	50	Control
1	0 0 0[a]	0 0 0	0 0 0	0 0 0	0 0 0	0 0 0	0 0 0	0 0 0	0 0 0
4	0 0 0	0 0 0	0 0 0	0 0 0	0 1 0	0 1 1	0 1 0	0 1 1	0 0 0
8	0 0 0	0 0 0	0 0 0	0 0 0	0 1 1	1 0 1	1 1 1	1 2 1	0 0 0
12	0 0 0	0 0 0	0 0 0	0 0 0	0 1 1	0 2 2	1 2 2	1 4 4	0 0 0
24	0 0 0	0 0 0	1 1 0	0 0 0	1 1 1	4 4 4	4 4 2	4 4 4	0 0 0
48	0 0 0	1 3 1	3 1 1	1 0 0	1 1 1	4 4 4	4 4 4	4 4 4	0 0 0
72	0 0 0	1 3 1	3 1 0	1 0 0	1 1 1	4 4 4	4 4 4	4 4 4	0 0 0

Key: 0—no symptoms.
1—primary wilting of leaflets and petioles.
2—complete wilting of leaves and stem.
3—chlorosis and/or necrosis; no wilting.
4—wilting and chlorosis and/or necrosis.

[a] Three cuttings in each test series.

move the proteinaceous fraction was atypical of that observed in naturally infected plants. Whereas occlusion of the vascular cylinder appeared to occur, none of the cuttings wilted. There seems to be little question but that the discoloration was the result of imbibition of the amber colored precipitate, identified as a polysaccharide fraction.

From the evidence presented it seems presumptive to assume that the toxic substances elaborated in vitro by *V. albo-atrum* are responsible for the plant responses observed under conditions of natural infection. If a toxin is, indeed, synthesized by the pathogen, it is likely to be an adaptive response and only a critical evaluation of the host-parasite relationship will yield conclusive evidence of such a substance.

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PRODUCTION AND PROPERTIES OF ANTIMYCIN A FROM A NEW STREPTOMYCES ISOLATE [1]

J. L. Lockwood,[2] Curt Leben, and G. W. Keitt

SUMMARY

A crystalline antifungal antibiotic, antimycin A-102, was isolated from cultures of *Streptomyces* sp. isolate 102. This product differs slightly from antimycin A-35, a previously reported crystalline antibiotic produced by a different *Streptomyces* isolate, No. 35. These antibiotics contain, in different proportions, 4 common active components, which have been named antimycins A_1, A_2, A_3, and A_4.

Antimycin A-102 has been produced in shaken flasks and 30-l. fermentors. The crystalline product was obtained by acid precipitation and solvent fractionation. Some of the chemical and physical properties of partly purified antimycin A-102 and crystalline antimycin A-102 are reported.

In agar-streak tests partly purified preparations and crystalline preparations reduced the growth of nearly all the fungi tested, but complete inhibition was usually not achieved even at high concentrations. Partly purified preparations were inhibitory to some bacteria whereas the crystalline product was not. The pH of the medium was shown to affect the level of inhibition of several fungi by partly purified or crystalline preparations of antimycin A-102. The pH at which greatest inhibition occurred was not the same for all fungi, and for some fungi bimodal inhibition was shown.

Aqueous suspensions of partly purified antimycin A-102 gave average ED 50 and ED 95 values of 23 and 297 μg. per ml., respectively, in greenhouse tomato early blight protectant tests. Fifty-five per cent of the active material in similar preparations remained on tomato foliage after washing with 2.5 cm. of simulated rain. The preparation was most resistant to washing at an acid pH. Seventy per cent of the activity remained on unwashed foliage after 4 days. Concentrations of 2.9 mg. per ml. applied as sprays were non-phytotoxic to several species of plants. In preliminary seed treatment tests in the greenhouse the partly purified antibiotic was effective in controlling a *Helminthosporium* seedling blight of oats.

On the basis of higher antibiotic yields and a simpler purification procedure isolate 102 appears to be a more satisfactory organism than the original organism, isolate 35, for the production of the antimycin A complex.

An antifungal antibiotic, named antimycin A-102 has been isolated in crystalline form from cultures of *Streptomyces* sp. isolate 102, an organism previously described (23) and designated A102 (25). At the outset and throughout most of this investigation, antimycin A-102 was assumed to be distinct from other antifungal antibiotics, but as research progressed the partly purified material appeared to resemble antimycin A, a crystalline antibiotic (5). However, isolate 102 differed from the original antimycin A organism, isolate 35, with respect to antibiotic spectrum and morphological and cultural characteristics (23, 26). Comparative tests made with both crystalline antibiotics showed the new antibiotic to be antimycin A, but to differ in certain particulars from that first described. Therefore the original antimycin A is designated antimycin A-35, whereas that produced by isolate 102 is called antimycin A-102. A brief report of some of this work has already appeared (14).

During the course of research both antibiotics were shown to contain 4 common components. For convenience, however, the term antibiotic is used in the singular when referring to this complex.

Antimycin A-35 has recently been shown specifically to block, at very high dilutions, a component of the hydrogen transport mechanism of higher, aerobic organisms (1, 17, 18). Progress has also been made in elucidating the chemical structure of antimycin A-35 (27, 28).

PRODUCTION, ASSAY, AND PURIFICATION OF ANTIMYCIN A-102.—*Production.*—In tests with several types of media in an earlier screening program (25), and also in the current investigation, *Streptomyces* sp. isolate 102 gave highest antibiotic yields when grown on a soybean meal-glucose medium.[3] This medium was used in all experiments reported herein. In most fermentations a preparation of 10 per cent DC Antifoam A (Dow-Corning Co., Midland, Mich.) in soybean oil was added.

Small scale fermentations were carried out in 2 or 4 l. Erlenmeyer flasks containing 500 or 1000 ml. of liquid medium, respectively, and 2 ml. of the anti-

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[3] Per l.: soybean meal, 40 gm.; glucose, 20 gm.; $CaCO_3$, 2 gm. The meal giving highest yields was a 44 per cent extracted meal obtained from the Archer-Daniels-Midland Co., Minneapolis, Minn.

TABLE 1.—*Fractionation procedure for antimycin A-102 and data for a sample fractionation*

Step	Treatment	Fraction tested	Total activity in units	Units per mg. dry wt.
1.	58 l. fermented medium[a] mixed with 9 kg. Celite 503; pumped through filter press precoated with Celite. Filter cake discarded.	Filtrate	6,290,000	8.2
2.	Filtrate (37 l.) adjusted to pH 3.5 with HCl; mixed with 2 kg. Celite; precipitate filtered through press. Inactive supernatant discarded.	.....	.....	.....
3.	Filter cake extracted by recycling 3, 6 l. portions of 95 per cent ethanol through press.	Extract	5,950,000	76
4.	Ethanol extract taken to 0.2 original volume (reduced pressure); 0.5 kg. Celite added and preparation taken to dryness. Residue extracted in Soxhlet extractor (50 flushes) with petroleum ether.[b] Residue discarded.	Extract	4,700,000	128
		Residue	1,100,000	.....
5.	Petroleum ether extract concentrated to small volume; cooled (4°). Precipitate recovered by filtration; washed with petroleum ether to remove inactive gum, leaving active brown powder.	Petroleum ether washes	1,960,000	.....
6.	Brown powder dissolved in minimum amount of ethyl ether; cooled (4°). Crystals recovered by filtration; washed with ethyl ether-petroleum ether mixture (2:1). Second and third crops of crystals recovered from mother liquor in same manner.	Crystalline product	2,200,000	2,000
		Mother liquor	300,000	.....

[a] pH 7.4. Harvested at 4 days.
[b] Skellysolve A, b.p. *ca.* 35°.

foam preparation per l. of medium. Flasks were shaken at 26°C. on a reciprocal shaker making 65 cycles per minute with a 15 cm. stroke and a 28 cm. radius. The medium was inoculated with spore suspensions prepared from 10–20 day-old slant cultures of the antibiotic organism grown on yeast extract-glucose agar (per l.: yeast extract, 2 gm.; glucose, 10 gm.; agar, 17 gm.) at 26°. Stock cultures of the organism were kept in soil at 4°. Maximum activity as determined by assays of culture filtrates or centrifugates was 50–85 arbitrary units per ml. (a unit is described below), and was achieved in 4–6 days.

Larger amounts of the antibiotic were produced in 30 l. stainless steel fermentors (19) containing 15 l. of medium and 150 ml. of the antifoam preparation. Fermentors were partly immersed in a water bath at 26°. Sterile air was passed through the medium at a rate of 0.5 volumes of air per volume of medium per minute. The medium in each jar was seeded with 1 l. of a 4–5 day shaken culture prepared as described above. Three runs of 3–4 fermentors each were made. The fermented medium was harvested after 3–4 days at which time centrifugates assayed 260–400 units per ml.

Assay.—Estimations of antibiotic potency were made by an agar diffusion assay (5). The fungus *Glomerella cingulata* (Stonem.) Spauld. & v. Schrenk was employed as the test organism, and the assays were carried out in large culture plates (15). The antibiotic preparations, which were carried in 80 or 100 per cent ethanol, were applied to the seeded agar in paper disks. The antimycin A-102 standard used throughout most of the work was a partly purified ethanol solution of the antibiotic. In later work crystalline antimycin A-35 (1 μg. = 1 unit) was used. All estimations of potency are given as antimycin A-35 units. Dosage-response curves for the standard and test preparations were usually straight lines and parallel.

The liquid portion of the cultures was used as the source of partly purified and crystalline antimycin A-102 for the work reported herein. Recent studies, not yet completed, indicate that additional amounts of the antibiotic are present in the solid portion of the cultures, and that this activity can be recovered by modification of the fractionation procedure.

Purification.—In preliminary experiments with culture centrifugates the active material was extracted with various solvents (e.g., *n*-butanol, ethyl ether, chloroform, and amyl acetate). Later it was found that the activity could be more conveniently obtained by ethanol extraction on the precipitate which formed when the culture centrifugate was adjusted to pH 3.5–4.0. With the aid of the assay a solvent fractionation procedure was developed which led to the recovery of an active crystalline product. The purification procedure and data for a sample fractionation of a fermentor culture are in Table 1. The fractionation procedure for shaken flask cultures was similar except that centrifugation or filtration with a large Buchner funnel was employed in the first 3 steps. For all experiments using partly purified material, ethanol solutions of dry petroleum ether extracts (Table 1, Step 4) were used.

SOME CHEMICAL AND PHYSICAL PROPERTIES.—Solubility and stability tests were made with partly purified preparations of antimycin A-102 containing 128 or 180 units per mg. To test solubility dried samples of an ethanol solution, each containing 1.2 mg. of solids and 2.5 units, were shaken with 5 ml. of the test sol-

vent. Assays of ethanol solutions of the dried supernatant liquids and the undissolved residues showed that the active material was soluble in ethanol (50 or 100 per cent), methanol, *n*-butanol, ethyl ether, amyl acetate, methyl acetate, chloroform, benzene, acetone, carbon tetrachloride, methyl cellosolve, and petroleum ether (Skellysolve A). The active material was probably soluble in 0.05 M phosphate buffer at pH 10, but instability at this pH made results equivocal. The antibiotic was partly soluble in ethylene glycol and 0.05 M phosphate buffer at pH 7.5; it was insoluble in 0.05 M phosphate buffer at pH 2.5 or 5. Ethanol solutions of partly purified antimycin A-102 diluted with several volumes of water produced fine suspensions, the pH values of which were usually 3.6–3.9.

Stability tests were carried out with aqueous suspensions of the antibiotic preparation at various temperatures and pH levels. Results showed that the antibiotic was most stable in the acid range, and was heat labile, especially in the alkaline range. Ethanol solutions at 4° and dried preparations at room temperature have retained all their activity after storage for 18 months. Culture centrifugates showed a marked decrease in activity after a few days at 4°.

A preparation of antimycin A-102 containing 425 units per mg. gave the following strong positive tests for phenols: $FeCl_3$, Folin, diazobenzene-sulfonic acid, and Gibb. The xanthoproteic test for an activated benzene ring was also positive. Negative Molisch, anthrone, Benedict, phenylhydrazene, ninhydrin, Hopkins-Cole, Millon, and biuret tests were given. Using the technique of Bennett et al. (3), a strong test for nitrogen, a weak test for sulfur, and negative tests for halogens, arsenic, and phosphorus were given. The same preparation in absolute ethanol gave essentially the same ultraviolet absorption spectrum as antimycin A-35 (27). These data, especially the uv spectrum, were the first to suggest that antimycin A-102 and antimycin A-35 were similar.

Crude crystalline, antimycin A-102, the product of the first crystallization, is purple, whereas the more highly purified product, obtained by recrystallization, is colorless. Crude crystalline preparations usually melted at 126–128°, but these preparations sometimes partly melted at approximately 126°, then did not melt further until a temperature of 144–146° had been reached. A highly purified product obtained by alternate recrystallization from ethyl ether and methanol melted at 148–149.5°. Melting points were found to depend on the temperature of the oil bath at the time of immersion of the sample: the warmer the bath, the lower were the melting points. All melting points reported herein for antimycin A-102 were carried out with the bath at an initial temperature of 50°. Tests showed that melting points of crystalline antimycin A-35 were not affected by the initial temperature of the bath.

A crystalline preparation in 95 per cent ethanol gave uv absorption maxima at 225 and 320 mμ. A bathochromic shift was noted on the addition of 2 equivalents of base.

EFFECT ON CERTAIN FUNGI AND BACTERIA IN VITRO.—The effect of partly purified antimycin A-102 on a number of fungi and bacteria was examined in 2 series of agar-streak tests using routine methods (10, 13). Two-fold dilutions of the antibiotic preparation (110 units per mg.) in ethanol were used.[4] The test organisms included 16 species of plant pathogenic fungi, for the most part those used in previous work (10); 5 yeasts; and 10 bacteria, 5 of which are plant pathogens.

The results of both series of tests were similar. Growth of nearly all the fungi, including the yeasts, was restricted by the antibiotic preparation at 0.025 μg. per ml. Since the preparation used contained only about 5 per cent antimycin A-102, the effects of the antibiotic were exerted at extremely high dilutions. Few fungi were completely inhibited, however, even at the highest concentration, 80 μg. per ml. Thus, partial inhibition was shown over a very wide concentration range—much wider than observed with helixin (13) and toximycin (24), for example. The growth of most of the bacteria was restricted at the highest concentrations, but the range of partial inhibition appeared to be narrower than with the fungi. These results are in contrast with those obtained for partly purified preparations of antimycin A-35, which gave definite end points and did not produce partial inhibition of fungi over a wide concentration range (10).

The effect of different pH levels on the activity of crystalline antimycin A-102 was studied in agar-streak tests using methods similar to those of Leben and Keitt (12).[4] Part of the data are in Table 2. The degree of inhibition of most fungi varied with the pH, but the pH level for greatest inhibition was not the same for all fungi. In some cases bimodal inhibition was shown, i.e., greater inhibition at pH 3.6 and 8.5 than at pH 6.9.

Similar experiments were carried out using the same organisms and partly purified preparations of antimycin A-102. The results were similar to those for the crystalline antibiotic, but some data were equivocal because the impure preparation altered the pH of the medium.

STUDIES ON PARTLY PURIFIED ANTIMYCIN A-102 IN RELATION TO PLANT DISEASE CONTROL.—*Early blight protectant tests.*—The protectant action of a partly purified preparation of antimycin A-102 was studied in 3 greenhouse tests with the early blight disease of tomato, incited by *A. solani*. The methods of McCallan and Wellman (16) as modified by Leben and Keitt (11) were used. An ethanol solution of the antibiotic (220 units per mg.) was diluted in distilled water to prepare the test suspensions. Three plants were sprayed with each concentration in each trial. A

[4] Controls showed that the ethanol in which the antibiotic was carried (highest concentration, 1 per cent) did not affect the growth of any of the organisms, and that the antibiotic preparations did not change the pH of the medium.

TABLE 2.—*Influence of pH of the medium on inhibition of various fungi by crystalline antimycin A-102. Agar streak tests*[a]

Microorganism	Growth rating[b] Control pH 3.6	Control pH 6.9	Control pH 8.5	Antimycin A-102, 16 μg./ml. pH 3.6	Antimycin A-102, 16 μg./ml. pH 6.9	Antimycin A-102, 16 μg./ml. pH 8.5
Alternaria solani (E. & M.) Jones & Grout	4	5	5	1	2	2
Candida lipolytica (Harrison) Diddens & Lodder	5	5	5	2	1;0	2;4
Glomerella cingulata	5;4	5	5	1;0	4;3	2;1
Helminthosporium sativum P. K. & B.	5	5	5	3;2	2;1	2;1
Streptomyces scabies[c] (Thaxt.) Waks. & Henrici	0	5	5	0	5	5
Ustilago striiformis (West.) Niessl	5;4	5	5	1	4	2;3

[a] Results based on 2 tests completed at different times. Where results differed, second figure refers to second test. The pH levels given are for 1 test only; those for the second test differed by ± 0.2 pH unit.

[b] 5 = maximum growth as determined from plates at pH 6.9 without antibiotic; 1 = trace growth.

[c] Results from 1 test only.

spray of 2 per cent ethanol, the content of the most concentrated antibiotic preparation, caused no injury and did not affect the disease. The pH of the test suspensions varied from 3.8 (highest concentration) to 5.6–6.1 (lowest concentration). Dosage-response curves were prepared on log-probability paper by plotting the percentage disease control given by the various concentrations. The resulting functions were straight lines.

The average ED50 value was 23 μg. per ml. (range: 16–38); the average ED90, 168 μg. per ml. (range: 90–300); and the average ED95, 297 μg. per ml. (range: 150–540). The slope of the dosage-response curve, based on average values was 0.14 (ED50/ED90) or 0.08 (ED50/ED95), indicating a fairly flat slope. Although the pH of the antibiotic preparations used in the early blight tests differed, it seemed unlikely that this would affect the results since the buffering capacity of the strongest preparation used (280 μg. per ml.) was no greater than that of 0.001 N H_3PO_4 over the range 3.6–6.2.

Resistance to washing by simulated rain.—The resistance of partly purified antimycin A-102 to washing by simulated rain was studied in the greenhouse using the washing apparatus and a leaf disk assay similar to that described by Leben and Keitt (11). *G. cingulata* was used as the assay organism. Tomato plants were sprayed with an aqueous suspension of the antibiotic (270 units per mg.) containing 286 μg. per ml. (pH 3.8), dried, and leaf disk samples removed for assay. Plants were then washed with the desired amount of "rain," dried, and leaf disks again removed for assay.

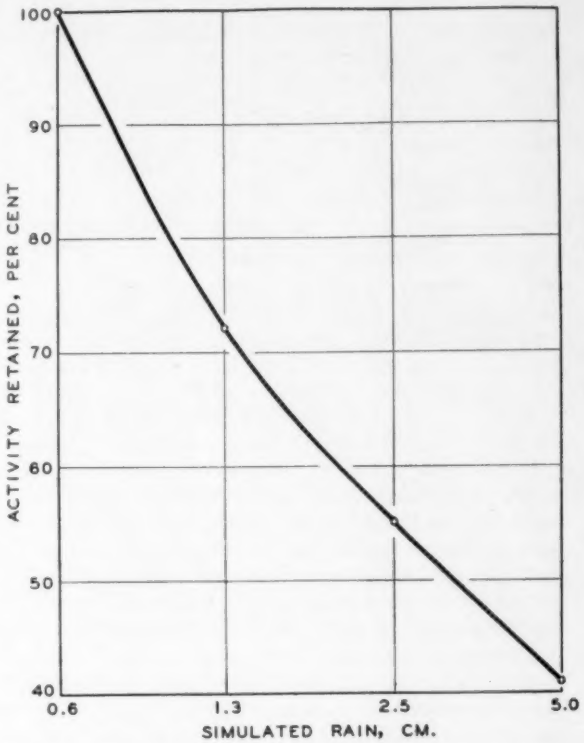

FIG. 1. Antibiotic activity remaining on tomato foliage after washing with simulated rain. Average of 3 experiments completed at different times.

Results showed that the preparation was resistant to washing (Fig. 1), and comparison of data from these experiments with those of Leben and Keitt (11) for partly purified and crystalline antimycin A-35 showed that the antimycin A-102 preparation was more resistant to washing. In another experiment plants were exposed to 2.5 cm. of simulated rain following application of the antibiotic preparation in 0.1 M phosphate buffers at pH 4, 7, or 10. After washing, the following percentages of activity remained: 75, <0.1, and 0, respectively. Results for the pH 10 formulation were equivocal, however, due to apparent instability of the antibiotic at this pH, since little activity was found on the leaves even before washing. In addition this formulation injured the leaves. It would appear that the partly purified antibiotic is most resistant to washing in the acid range.

Stability on the leaf.—The stability of partly purified antimycin A-102 on tomato foliage in the greenhouse was assessed by means of the leaf disk assay. Leaf disk samples were taken at various intervals from plants which had been sprayed with an aqueous suspension containing 270 units per mg. (pH 3.8). In 5 tests an average of 70 per cent of the original activity remained after 1 day, 67 per cent after 2 days, and 70 per cent after 4 days. In 1 experiment with a preparation containing 178 units per mg., leaf disks assayed after 8 days showed 62 per cent of the original activ-

TABLE 3.—*Effect of partly purified antimycin A-102 as a seed treatment material for the control of Helminthosporium seedling blights of barley and oats*

Test	Blight of:	Mg./gm. of seed: Antimycin A-102[a]	Mg./gm. of seed: Ceresan M[b]	Per cent seedling blight: Antimycin A-102	Per cent seedling blight: Ceresan M	Per cent seedling blight: No fungicide
1	Barley	0.55	1.3	61.3	12.7	92.2
	Oats	0.7	0.9	8.8	3.6	32.6
2	Oats	0.7	0.9	1.5	1.0	8.0
	Oats	0.14	—	1.5	—	—
	Oats	0.028	—	0.5	—	—

[a] Antibiotic preparation for test 1 contained 110 units per mg.; preparation for test 2 contained 178 units per mg.
[b] Amounts used are equivalent to 0.5 oz. per bu.

ity. It is concluded that the preparation is relatively stable on the plant.

Phytotoxicity studies.—An aqueous suspension of a partly purified preparation of antimycin A-102 (270 units per mg.) containing 2.9 mg. per ml. (pH 3.2) was sprayed on the foliage of several species of young plants in the greenhouse. The plants used were barley (*Hordeum vulgare* L.), corn (*Zea mays* L.), oats (*Avena sativa* L.), and wheat (*Triticum aestivum* L.), red clover (*Trifolium pratense* L.), bean (*Phaseolus vulgaris* L.), cabbage (*Brassica oleracea* var. *capitata* L.), cucumber (*Cucumus sativus* L.), pea (*Pisum sativum* L.), radish (*Raphanus sativus* L.), spinach (*Spinacia oleracea* L.), and tomato (*Lycopersicon esculentum* Mill.). Periodic examinations made for 1 month after treatment revealed no deleterious effects.

Seed treatment tests.—Partly purified preparations of antimycin A-102 in ethanol were tested as seed treatment materials for the control of the seedling blights of barley and oats, incited by *H. sativum* and *H. victoriae* M. and M., respectively. Naturally infested Wisconsin Barbless barley and Vicland oat seed were used, and experiments were carried out with the methods used by Leben et al. (8) for greenhouse tests with these diseases. There was no injury from the antibiotic preparations, and tests showed that ethanol as used in these experiments had no effect on the germination of seed or disease incidence. Partly purified antimycin A-102 was effective in reducing the amount of seedling infection, especially in oats (Table 3). Pertinent to these studies are those of Leben et al. (9), who tested crystalline antimycin A-35 as a seed treatment material for the control of the *Helminthosporium* blight of oats. In 3 greenhouse experiments, this material applied in ethanol at 1 concentration, 2.4 mg. per gm. of seed, was somewhat less effective than Ceresan M in controlling the disease.

Partly purified antimycin A-102 was tested as a corn seed protectant against *Pythium* spp. using the paper-doll technique of Hoppe (6). A preparation containing 178 units per mg., when applied in ethanol or as a dust at the rate of 0.3 mg. per gm. of seed, gave no disease control.

COMPARISON OF ANTIMYCIN A-102 AND ANTIMYCIN A-35.—The fact that *Streptomyces* sp. isolate 102 produces antimycin A was not suggested by earlier work nor by most of the studies using partly purified preparations. Furthermore, previous tests (23, 26) had shown that the *Streptomyces* spp. producing antimycins A-102 and A-35 were quite different in morphological and cultural characteristics on certain media (23, 26). Results of the analytical color tests and the uv spectrum determination made with partly purified preparations from isolate 102 indicated a strong similarity to antimycin A (5). Since the uv spectrum, especially, is a good criterion for characterizing a compound, it was deemed desirable to study the 2 antibiotics further in an attempt to establish their difference or identity.

Melting point and ultraviolet absorption spectrum determinations.—Comparison of the melting point of crude crystalline antimycin A-102 with that reported for crude crystalline antimycin A-35 (5) showed that both melted in the same range. The melting point of a preparation of antimycin A-102 alternately recrystallized from petroleum ether (Skellysolve A) and absolute methanol was 7–10° higher than preparations of antimycin A-35 repeatedly recrystallized from absolute methanol, ethyl ether, or other solvents to a constant melting point (5, 20). Mixed melting points of recrystallized preparations of both antibiotics showed no depression, however.

A crystalline preparation of antimycin A-102 in 95 per cent ethanol gave the same uv absorption maxima as antimycin A-35 (5), i.e., at 225 and 320 mμ., and, as with antimycin A-35, a bathochromic shift was noted on the addition of 2 equivalents of base (27).

Agar-streak tests.—To compare the inhibitory effects of the 2 crystalline antibiotics, agar-streak tests with several fungi and bacteria were made. Equal concentrations of antimycins A-102 and A-35 were employed. In the first test the range of final concentrations was 0.31–40 μg. per ml., and in the second it was 0.16–160 μg. per ml.[4] A portion of the data is in Table 4.

Each fungus was inhibited to essentially the same degree by both antibiotics, but the bacteria and *S. scabies* were not inhibited by either. Thus, the 2 products were indistinguishable in these tests. Partial inhibition was shown by both antibiotics over a wide concentration range.

The fact that crystalline antimycin A-102 showed no

TABLE 4.—*Comparison of the effect of crystalline antimycin A-102 and antimycin A-35 on several fungi and bacteria. Agar-streak tests*[a]

Microorganism	Growth rating[b]			
	Antimycin A-102		Antimycin A-35	
	40 μg./ml.	0.6 μg./ml.	40 μg./ml.	0.6 μg./ml.
Alternaria solani	3	4;3	4;3	4;3
Bacillus subtilis Cohn *emend.* Prazmowski	5	5	5	5
Candida lipolytica	1;0	2;1	1;0	2;1
Colletotrichum circinans (Berk.) Vogl.	2;1	2;1	2;1	2;1
C. lagenarium (Pass.) Ell. & Halst.	2	4;2	2	4;2
Corynebacterium fasciens (Tilford) Dows.	5	5	5	5
Erwinia amylovora (Burr.) Winsl. *et al.* No. 375	5	5	5	5
Erwinia amylovora (Burr.) Winsl. *et al.* No. 376	5	5	5	5
Erwinia caratovora (L. R. Jones) Holland	5	5	5	5
Fusarium oxysporum f. *pisi* (Linford) S. & H.	4;5	5	4;5	5
Glomerella cingulata	2;4	4	2;4	4
Helminthosporium victoriae	3	4;3	3	4;3
Stemphyllium sarcinaeforme (Cav.) Wilts.	0	2;0	0	2;0
Streptomyces scabies	5	5	5	5
Verticillium albo-atrum Reinke & Berth.	1;2	3;5	2;1	3;5

[a] Results based on 2 tests completed at different times; where tests differed, second figure refers to second test.
[b] 5 = maximum growth as determined by growth on plates without antibiotic; 1 = trace growth.

inhibitory action on any of the bacteria or on *S. scabies* is of interest, since the partly purified antibiotic was active against these organisms. The factor or factors responsible for this action were apparently lost in subsequent purification steps. It is possible that the differences in antibiotic spectra observed in the screening tests with isolates 102 and 35 may be explained by the production of antibiotic factors other than antimycin A.

Activity as determined by the agar diffusion assay.— The assay revealed a difference between the 2 antibiotics. Preparations of crystalline antimycin A-102 of varying melting points all demonstrated approximately twice the activity of antimycin A-35 (2 *vs.* 1 unit per μg.), although the dosage-response curves for both materials were parallel. Differences in potency were not detected in agar-streak tests (Table 4), but this may have been due to the level of accuracy attainable in these tests. It is possible, also, that the dissimilarity was due to a difference in diffusion rate rather than in innate toxicity, since the 2 antibiotics are composed of different proportions of 4 common components and the properties of the individual components are unknown (see below).

Paper chromatography.— Paper chromatography studies were made as a means of comparing antimycins A-102 and A-35. Ascending chromatograms were run on Eaton-Dikeman No. 613 filter paper strips 35.6 cm. long and 6.35 or 12.7 mm. wide. For each experiment 1–6 such strips were spotted with the antibiotic preparation, dried, and suspended in a sealed chamber, the atmosphere of which had been previously saturated with the developing mixture. The mixture used contained 62 per cent water, 33 per cent absolute ethanol, and 5 per cent glacial acetic acid, by volume. After equilibration for 3 hours, development was allowed to proceed by upward flow of the developing mixture until the solvent front had nearly reached the top of the strips (12–15 hours). Assays were made by laying the dried strips on seeded assay medium in large culture plates (15). Strips were removed from the agar 6–12 hours before assays were read so that zones of inhibition underneath could be detected. Absolute ethanol solutions of crystalline preparations and 50 per cent ethanol solutions of culture centrifugates were used. Crystalline antimycin A-102 was obtained from fermentor or shaken cultures. Crystalline antimycin A-35 was obtained from a fermentor culture using the medium given in footnote 3, except that the meal used was an extracted soy flour, type F, obtained from the A. E. Staley Co., Decatur, Ill. Culture centrifugates of antimycin A-102 were prepared from shaken cultures. Culture centrifugates of antimycin A-35 were prepared from shaken cultures produced from the same medium used to obtain the crystalline antibiotic. Chromatograms were developed at 30°.

In qualitative tests for the determination of zone positions, developed strips 12.7 mm. wide containing 0.085 units were assayed intact. When quantitative estimations of the activity of each component were desired, methods similar to those of Karnovsky and Johnson (7) were employed. Strips 6.35 mm. wide containing 0.43 units were used. After development these were cut into 6.35 mm. squares, which were assayed individually. Known amounts of the antibiotics in similar, dried squares were used for preparing a standard curve.

Results of the chromatographic studies may be summarized as follows: 1) Culture centrifugates and crystalline preparations obtained with both organisms were shown to contain 4 common active components, which have been designated (14) antimycins A_1, A_2, A_3, and A_4 (Fig. 2). The culture centrifugate of antimycin A-35 sometimes showed a 5th component (Table 5). Collectively these components are referred to as the "antimycin A complex." 2) In qualitative tests antimycin A-102 and antimycin A-35 appeared to possess the 4 components in different proportions. Quantitative studies carried out to examine this point showed that a difference does exist (Table 5). 3) The proportions of the components differed in culture centrifugates and crystalline preparations of the same antibiotic, probably reflecting disproportionate losses during purification (Table 5).

In qualitative tests crystalline preparations of antimycin A-102 of differing melting points from shaken

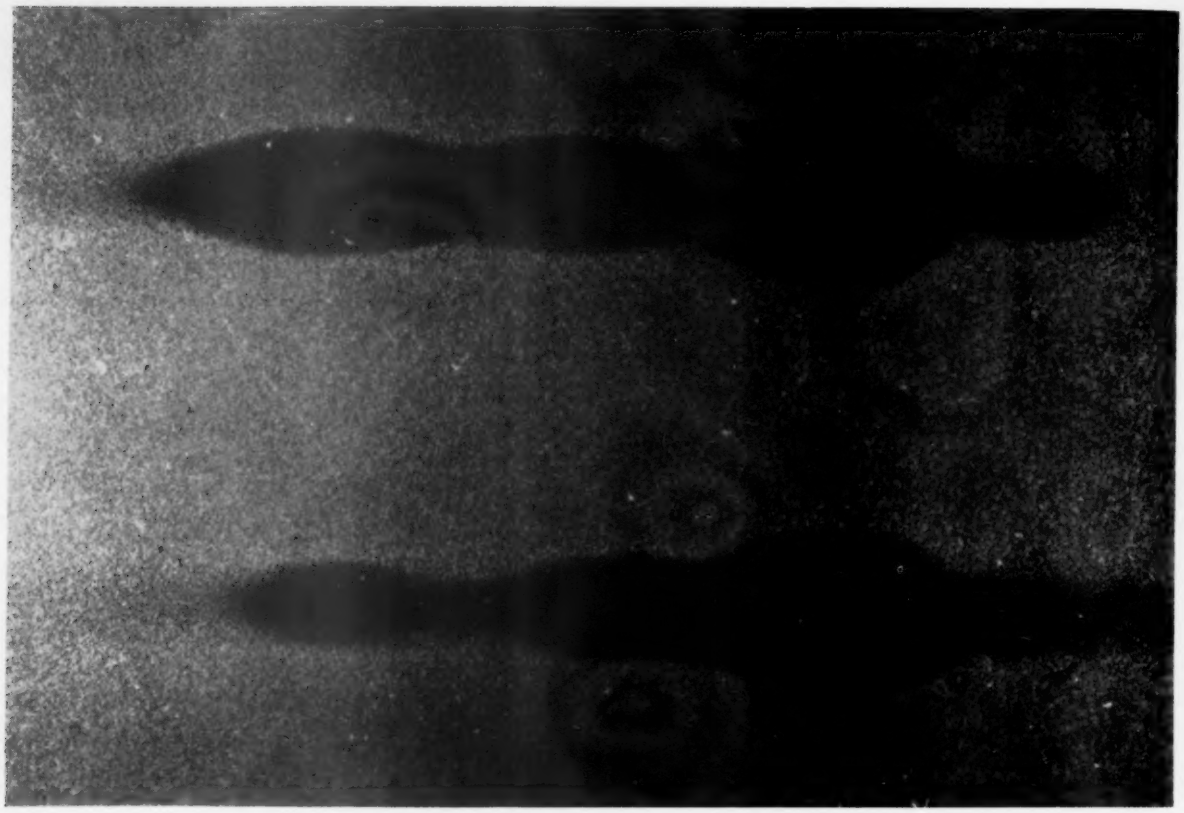

FIG. 2. Assay of paper chromatograms of crystalline antimycin A-102 and antimycin A-35. Top: antimycin A-35. Bottom: antimycin A-102. Left to right: antimycins A_1, A_2, A_3, and A_4, in order of increasing R_f values.

or fermentor cultures, and partly purified preparations from shaken cultures (Table 1, Step 4), all appeared to possess the 4 components in the same proportion. Thus the later stages of purification and recrystallization did not seem to alter the proportion.

The character of the inhibition zones produced by the 4 components differed. Antimycin A_1 gave zones with sharply defined edges, and with little growth of the assay fungus within the zones, whereas those of antimycin A_4 were quite faint, and without a definite margin. Zones produced by antimycins A_2 and A_3 were intermediate. Smith (22) has reported the resolution of antimycin A-35 into 3 components using a developing mixture similar to that used in the present study. His failure to report the fourth component was probably due to his not removing the paper strips from the agar plates before reading the assays.

The total activity of the 4 components on each strip agreed well with the amount applied. How closely the percentages of each represent the actual percentage composition by weight is not known since the characteristics of individual components are unknown, and certain properties (e.g., slope and position of dosage-response curve; rate of diffusion) would materially affect the values obtained.

To determine whether the 4 zones of inhibition might be due to spurious results, developed chromatograms of both crystalline antibiotics were cut into 4 pieces corresponding to the usual positions of the 4 zones, and the activity on each piece was eluted with ethanol. On assay, rechromatographed eluates usually gave 1 zone each, with an R_f value corresponding to that of the component eluted. Traces of the other zones were sometimes evident, especially with antimycin A_4. Thus, it would appear that the 4 zones of inhibition represent 4 distinct components present in the crystalline products, though it is possible that there was a small amount of interconversion of these components.

It is of interest that a highly purified crystalline preparation of antimycin A-35 (m.p. 149.8–150.2°) obtained from a silicic acid partition chromatographic column (20) was found to be almost entirely composed of antimycin A_1, when chromatographed as above. Faint traces of antimycins A_2 and A_3 were also evident. The slope of the dosage-response curve produced by this material in the agar diffusion assay differed from that of crystalline antimycin A-35, which contains all 4 components.

Whether the difference in the proportions of the components in the 2 crystalline materials is a function of the organisms, purification procedures, or of

TABLE 5.—*Percentages of activity and R_f values of the components in culture centrifugates and crystalline preparations of antimycin A-35 and antimycin A-102, as determined by the agar diffusion assay*[a]

	R_f value				Per cent of activity			
	Culture centrifugate		Crystalline product		Culture centrifugate		Crystalline product	
	Antimycin A-		Antimycin A-		Antimycin A-		Antimycin A-	
Antimycin	35	102	35	102	35	102	35	102
A_1	0.32	0.31	0.31	0.35	15	22	31	17
A_2	0.44	0.46	0.45	0.50	36	26	16	21
A_3	0.58	0.59	0.56	0.63	26	37	50	56
A_4	0.68	0.75	0.67	0.74	23	15	3	6
A_5	0.78	—	—	—	—	—	—	—

[a] R_f values average of 4–6 trials; percentages of activity average of 3 trials.

fermentation conditions has not been ascertained. Further, the extent and significance of differences in the properties of the individual components is also to be determined. The essentially identical results of agar-streak tests for the 2 crystalline antibiotics, and the parallel slopes of the assay dosage-response curves suggest that the 4 components act in a similar, if not identical manner. Indeed they must be very similar chemically, inasmuch as repeatedly recrystallized antimycin A-102 contained the 4 components in apparently the same proportion as did the partly purified preparation and the crude crystalline product. On the other hand, differences in the character of the inhibition zones produced by the different components, the greater activity of antimycin A-102 as determined by the assay, and the fact that the slope of the assay dosage-response curve for antimycin A_1 is not the same as that of the complex, suggest that the differences in the components may be of sufficient order that certain combinations may differ considerably in their properties. Until all the components are separated and studied individually final answers to such questions cannot be given.

Paper chromatograms of partly purified antimycin A-102 were made using water-saturated benzene as the developing mixture. Assays showed a very small zone at the point of application of the antibiotic and a large zone at the solvent front. These zones corresponded in relative size and position to antimycins A (large, mobile component) and B (smaller, nonmobile component) as defined by Schneider et al. (20) using partly purified antimycin A-35 and a similar developing mixture. It is probable, therefore, that isolate 102 produces antimycin B in addition to the antimycin A complex.

From the experiments reported in this section, it is concluded that antimycin A-102 is very similar to antimycin A-35, yet differs slightly from it in certain respects. It would appear that the differences are a function of a dissimilar proportion of the 4 components in each antibiotic.

DISCUSSION.—The production of the same antibiotic by more than 1 *Streptomyces* species is not uncommon (2). This fact and our experience with antimycin serve to emphasize the importance of employing definitive methods for distinguishing and characterizing antibiotics early in a screening program.

The inability of antimycin A to inhibit completely the growth of most fungi *in vitro*, even at high concentrations, is analogous to the findings of Potter and Reif, who studied the action of crystalline antimycin A-35 on oxidative enzyme systems by manometric means (17, 18). These workers found antimycin A-35 to be a highly selective and potent inhibitor of an electron transport component, probably the "Slater factor," which acts between succinic dehydrogenase and cytochrome c in the succinoxidase system (4), and between diaphorase and cytochrome c in DPN systems (4, 21). The succinoxidase system in various rat tissues was always completely inhibited by the antibiotic. Certain of these tissues, however, when tested as DPN-oxidizing systems, showed only partial inhibition of oxygen uptake at the same levels of antimycin A as were required for complete inhibition of the succinoxidase system. Moreover, the degree of inhibition could not be increased, even at very high concentrations of antimycin A-35. The residual respiration ranged from 19-97 per cent, depending on the tissue. These findings suggested that there are 2 pathways for oxidizing reduced DPN—antimycin A-sensitive and antimycin A-insensitive—and that the ratio between them varies from tissue to tissue. If respiration proceeds in a similar fashion in the fungi, it is quite possible that the same or other antimycin A-insensitive pathways may account for the apparent inability of antimycin A to inhibit completely the growth of many fungi.

Antimycins A-35 and A-102 have not been directly compared in plant disease control experiments, but a comparison of results reported herein for partly purified antimycin A-102 with data for partly purified antimycin A-35 (11) indicates that the 2 materials are similar with respect to slope and position of the dosage-response curve in early blight protectant tests, and with respect to stability of the active material on tomato foliage. The antibiotics appear to differ in that preparations of antimycin A-102 on tomato foliage were more resistant to washing by simulated rain.

Streptomyces sp. isolate 102 may prove to be a more favorable organism for the production of the

antimycin A complex than isolate 35. Fermented cultures of isolate 102 fractionated as in Table 1 yielded about twice as much crystalline product as did similar fermentations of isolate 35 using the fractionation scheme of Dunshee et al. (5), as modified by Schneider et al. (20). A considerable advantage of isolate 102 is the shorter fractionation procedure that can be used.

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THE RENIFORM NEMATODE AND ITS RELATIONSHIP TO THE INCIDENCE OF FUSARIUM WILT OF COTTON AT BATON ROUGE, LOUISIANA

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SUMMARY

High incidence of Fusarium wilt in a susceptible variety of cotton in the Baton Rouge area is dependent upon the presence in the soil of a heavy population of the reniform nematode. Several plots at the Experiment Station Farm near Baton Rouge are moderately to heavily infested. The parasite is, however, strikingly localized, and, so far as known, does not occur in other wilt-infested districts of the state outside of East Baton Rouge Parish.

A greenhouse technique has been developed to screen cotton varieties for resistance or susceptibility to the disease complex within 6–7 weeks. It requires that plants be grown in soil infested with the wilt fungus and a nematode population comparable to that present in areas where wilt damage is severe.

The reniform species apparently is unable to break the wilt resistance of certain strains of cotton developed in this area and at other localities where root-knot nematode and wilt prevail. However, recent tests in Alabama reveal that certain reniform-wilt-resistant strains are susceptible to wilt where this disease and the root-knot nematode are associated. In addition, several reniform wilt-resistant strains tested at Natchitoches, Louisiana, in 1953 in an area heavily infested with root-knot nematode and wilt were, with 3 exceptions, susceptible to this disease complex.

This paper reports the results of soil examinations of Fusarium wilt-infested-cotton plots at the Perkins Road Experiment Station Farm near Baton Rouge to determine the presence of parasitic nematodes and their importance in the prevalence of the disease. The reniform nematode, *Rotylenchulus reniformis*, was recovered from these plots in large numbers and other parasitic species to a lesser extent. For ascertaining the importance of the reniform nematode in the prevalence of wilt in this area, a greenhouse-infection-technique was developed. A description of this technique is given. Also, certain strains and varieties of cotton which have been bred and tested for resistance under reniform-nematode-wilt infestation are mentioned together with their behavior at localities where the important root-knot nematode, *Meloidogyne incognita var. acrita*, occurs with wilt.

Rotylenchulus reniformis, was first reported as a plant parasite in Hawaii in 1940 by Linford and Oliveria (2). They mentioned that the species was seen as early as 1935, but "as the parasite appeared to be of minor importance, no detailed study was made". Linford and Yap (3) also published in 1940 a list of some of the host plants of this nematode. They gave the results of testing 65 hosts representing 30 families. Several plants were noted as resistant. Cotton was not mentioned.

In 1940, Smith (8) stated, "there was also a heavy infestation of a form which Steiner has identified as representing the new species being described from Hawaii by Linford". Since that time Steiner has advised the writer that the form referred to by Smith near Cuthbert, Georgia, was indeed *Rotylenchulus*, but that it may not have been *Rotylenchulus reniformis*, but a related species. The importance of this *Rotylenchulus* species in relation to wilt in Georgia was not determined. In 1941, Smith and Taylor (9) reported that a pronounced infestation of the reniform nematode occurred in the regional wilt plots on the roots of both cotton and cowpeas collected from Baton Rouge. These authors no doubt are referring to the wilt plots formerly located at the Old Station Farm which was discontinued in 1942. This is the first published record of this species having been found on cotton in association with Fusarium wilt. Further observations on the presence of this nematode in wilt-infested soil were reported in 1953 (6). On November 18, 1952, composite soil samples were obtained at horizons ranging from 3–9 in. from heavily wilt-infested cotton plots at the Perkins Road Experiment Station Farm near Baton Rouge. Half portions of these samples were forwarded to A. L. Taylor, Nematologist, Division of Nematology, Beltsville, Maryland, for analyses of the nematode population. The predominant form found by Taylor in this soil was *Rotylenchulus reniformis*, the reniform nematode. *Helicotylenchus nannus*, another parasitic form, also was present but in smaller number.

Also, in nematode counts of the same samples made by Wray Birchfield, Graduate Research Assistant, Louisiana Experiment Station, 345 gm. of this soil, approximately ¾ of a pint, had a reniform nematode population in excess of 6500. This is considered by nematologists a heavy population of a parasitic form for crop plants. In June 1953 the writer also obtained soil samples from the Old Station wilt plots mentioned above and referred them to W. J. Martin, Associate Plant Pathologist of the Louisiana Station, for nematode examination. He recovered *Rotylenchulus reniformis* from this soil. There are, therefore, 2 locations in this vicinity, the Old Station Farm and the Perkins Road Station Farm, separated from each other by

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about 5½ miles, which harbor this nematode in wilt-infested areas.

In view of the established prevalence of this nematode in the wilt plots of Baton Rouge, subsequent studies were made to determine to what extent it contributes to the high incidence of wilt at this locality, where infection in a susceptible variety like Half and Half is frequently as much as 95 per cent. Since certain strains of cotton selected by the writer in the above area have exhibited a high degree of resistance to this nematode-wilt complex in trials from 1946 to 1952 inclusive, it was of interest to know whether their field behavior could be attributed to possession of genes for resistance to the dual effect of the parasite in association with the wilt fungus, and whether the resistance of these strains is maintained when grown at other localities where the important root-knot nematode occurs with wilt.

MATERIALS AND METHODS. — Greenhouse-infection techniques as well as field-infection techniques, for studying Fusarium-wilt resistance in cotton have been reported by several investigators (1, 4, 5, 6, 7, 10). In the present study, 2 large flats measuring approximately (inside dimensions net) 35¾ in. long, 32¼ in. wide, and 9¼ in. deep, were used to grow a wilt resistant and a wilt susceptible variety of cotton in the greenhouse. On November 25, a quantity of Olivier silt loam was obtained from the plots which showed upon analysis, in November, the heavy reniform nematode population previously mentioned.

The infested soil was screened to remove grass, roots, and other debris and later placed into each of the 2 flats, to within 3½ in. of the tops. Each flat contained approximately 4 cu. ft. of soil. One was kept in subdued light and covered with paper to prevent drying out and the other was placed in a sterilizer and autoclaved for 2 hours at 30 lbs. pressure. On the fifth day following sterilization of the soil, magnesia lime was added to both flats at the rate of 2000 lbs. per acre to increase the soil pH and 1000 lbs. per acre of a 5-10-5 fertilizer, N-P-K, to promote normal growth of the cotton, was also added. Meanwhile, the fungus contents of twelve 250-cc flask cultures of the wilt fungus in Czapek's solution, 36 days old, were washed in 3 changes of sterile distilled water to remove traces of possible staling products and later chopped and blended for 10 minutes for uniformity in a Waring blendor. To this mixture was added a heavy spore suspension of the wilt fungus, *Fusarium oxysporum f. vasinfectum*, prepared from 10 fresh agar-slant cultures of an isolate of the fungus obtained from the infested areas on October 20. This inoculum was mixed thoroughly with the soil, 1 liter per flat, and the moisture content of the soil adjusted to approximately 65 per cent of its water-holding capacity. Plantings were made on December 16, 1952, one half of each flat being seeded to the highly wilt-resistant variety, Delfos 425-920, and the other half to the highly susceptible variety, Half and Half. Also, to aid in maintaining a favorable level of the nematode population, the flat containing naturally infested soil was seeded lightly with hairy vetch.

Emergence of seedlings was good and only moderate loss occurred due to *Rhizoctonia solani*. The temperature of the greenhouse during the course of the experiment fluctuated from approximately 74 to 85°F. Wilt readings, including plants infected and killed, were recorded for the first time on January 24, 39 days after planting, and thereafter at 6, 7, and 12-day intervals for a total growth period of 99 days. At this time soil samples were obtained from each flat by L. D. Newsom, Associate Entomologist of the Louisiana Experiment Station, and analyzed for nematode population. The naturally infested soil plus artificial wilt inoculation had 4370 reniform nematodes per pint of soil, 160 spiral forms, *Helicotylenchus sp.*, and many saprophytic and predatory forms. The sterilized soil reinoculated with wilt culture had only 30 of the reniform species and very few saprophytes and predatory forms for the same unit of soil. The experiment was terminated on March 25, 1953, and the results are shown in Table 1.

DISCUSSION.—In the flat containing soil naturally infested with both wilt and a heavy nematode population and also given artificial-wilt inoculation, development of the disease in Half and Half, the susceptible variety, was highly significant, the accumulative wilt-infection reaching 81.4 per cent at the termination of the experiment.

No disease developed in the resistant variety, Delfos 425–920, growing in the naturally infested soil until February 19th, 59 days after planting. On this date only 3.1 per cent of the plants were wilted and no additional plants showed wilt for the duration of the experiment. (Fig. 1A) In the flat containing sterilized, nematode-free soil and subsequently artificially inoculated with wilt cultures, the susceptible variety developed only 10 per cent infection, whereas none of the plants of the resistant variety in this flat became infected. (Fig. 1B)

The results of this experiment indicate definitely that the presence of the reniform nematode is an important factor in the incidence of wilt in this area, especially where susceptible varieties are concerned. Field trials on the heavily infested fields of the Old Station Farm and the Perkins Road Experiment Station have shown that other varieties of cotton developed in Louisiana and elsewhere are also able to withstand the combined attacks of Fusarium and reniform nematodes. Among these are Auburn 56 and Plains from Alabama; Delfos 425–920, Delfos 9169, Deltapine 6, Deltapine 6 × Delfos 6102, Cook 307-6 × Delfos 9169, Louisiana Hybrid 33 × 14-312, Louisiana 33, Roxie, and Stoneville 2B strains from Louisiana; and Coker 100 Wilt and derivatives from South Carolina.

Reports from Alabama, however, reveal that the reniform-wilt-resistant strains, namely, Delfos 425–920 and Louisiana Hybrid 33 × 14-312 were wilt-suscepti-

FIG. 1. A) Cotton plants grown for 100 days in Olivier silt loam naturally infested with Fusarium wilt and reniform nematodes and also given artificial wilt inoculation. Variety at left of A, Delfos 425–920 (wilt-resistant), wilt infection 3.1 per cent; variety at right of B, Half and Half (wilt-susceptible), wilt infection 81.4 per cent. B) Same varieties also grown for 100 days in sterilized, Olivier silt loam and reinoculated with Fusarium wilt only. Variety at left of A, Delfos 425–920 (wilt-resistant), wilt infection, none; variety at right of B, Half and Half (wilt-susceptible), wilt infection, 10 per cent. Photographed March 26, 1953.

TABLE 1.—*Reaction of a resistant and a susceptible variety of cotton grown for 99 days in soil naturally infested with Fusarium wilt and reniform nematodes and given artificial wilt inoculation and in sterilized soil reinoculated with the fungus*

(Flat 1)

Naturally infested soil plus artificial wilt inoculation

Variety	Number of Plants	Percentage wilt infection at dates indicated								
		1/24	1/31	2/6	2/13	2/19	2/26	3/5	3/12	3/25
Delfos 425-920	32	0	0	0	0	3.1	3.1	3.1	3.1	3.1
Half & Half	27	11.1	22.2	29.6	33.3	37.0	40.7	51.8	59.2	81.4
		Number killed at dates indicated								
Delfos 425-920	—	0	0	0	0	0	0	0	0	0
Half & Half	—	0	2	1	5	2	3	1	3	0

Planted Dec. 16, 1952

Total killed:
Delfos 425-920, none
Half & Half, 17

Soil type:
Olivier silt loam

(Flat 2)

Sterilized soil re-inoculated with wilt culture

Variety	Number of Plants	Per cent infection at dates indicated								
		1/24	1/31	2/6	2/13	2/19	2/26	3/5	3/12	3/25
Delfos 425-920	38	0	0	0	0	0	0	0	0	0
Half & Half	30	0	0	3.3	3.3	3.3	6.6	6.6	6.6	10.0
		Number killed at dates indicated								
Delfos 425-920	—	0	0	0	0	0	0	0	0	0
Half & Half	—	0	0	0	1	0	1	0	0	0

Total killed:
Delfos 425-920, none
Half & Half, 2

ble in that state where the root-knot nematode and wilt are associated.

Of a total of 22 strains resistant to wilt under conditions of reniform-nematode-wilt infestation at Baton Rouge, all were, with few exceptions, susceptible in tests conducted the past season at Natchitoches, Louisiana, in Yahola Very Fine Sandy Loam heavily infested with root-knot nematodes and wilt. The exceptions were Coker 100–70 Ga., which had 26.2 per cent infection on September 23 as compared to 23.6 per cent for Coker 100 Wilt and 24.9 per cent for Plains, the last 2 being wilt-resistant commercial varieties developed in Alabama and South Carolina; and 2 strains of Stonesville 2B from Louisiana with 27.3 and 29.1 per cent respectively.

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PATHOGENICITY OF LEPTODISCUS TERRESTRIS ON RED CLOVER AND OTHER LEGUMINOSAE [1]

J. W. Gerdemann

SUMMARY

Leptodiscus terrestris has been found associated with a root rot of red clover, soybean, birdsfoot trefoil, and alfalfa. The fungus can be recognized in the field by its characteristic sclerotia. In culture, sporulation has been obtained only on natural media incubated in light. The optimum temperature for growth in culture was 30° C. with only a trace of growth at 10° or 35° C. In pathogenicity tests it caused damping-off and root rot of red clover, crimson clover, Ladino clover, white clover, alsike clover, birdsfoot trefoil, lespedeza, sweetclover, alfalfa, soybean, and pea. Only the root rot phase occurred on green bean and lima bean. The fungus penetrates living roots directly and wounds are not required for infection. No symptoms were produced on oats, wheat, corn, or cucumber. Leaf spots developed when spores were sprayed on leaves of soybean, red clover and alfalfa. Germinating spores produced appressoria and penetration of the host was direct. When seeded in warm, infested soil, the red clover varieties Midland, Kenland, and Dollard were completely susceptible to damping-off. When seeded in cold, infested soil, little damping-off occurred in any variety. When these plants became too old to damp-off, the soil temperature was raised and plants were killed by root rot. Kenland was most resistant, Midland intermediate, and Dollard most susceptible.

Leptodiscus terrestris Gerdemann (2) frequently has been isolated from diseased red clover, *Trifolium pratense* L., roots collected in southern and central Illinois as far north as Peoria. It also was isolated from decayed red clover roots collected at Beltsville, Maryland. It has been found on soybean, *Glycine max* (L.) Merrill, in central and southern Illinois, causing a disease very similar to Rhizoctonia root and basal stem rot. It was found associated with a root rot of birdsfoot trefoil, *Lotus corniculatus* L., at the Illinois Agricultural Experiment Station, Urbana, Illinois, and in personal correspondence, Dr. R. G. Henderson reported isolating it from roots of diseased alfalfa, *Medicago sativa* L., in Virginia. Attempts have not been made to find it on other crop plants; however, host range experiments indicate that it probably causes diseases of other legumes.

THE ORGANISM.—The fungus can be isolated by plating tissue from the advancing margin of decay, or sclerotia, on acidified potato dextrose agar or other types of agar media. Its growth on agar is appressed, at first hyaline becoming black as sclerotia form. Its final appearance is a thin black crust composed of many small sclerotia. Sporulation has not been obtained on agar media, nor has it been found in the field. Abundant sporulation occurs in the greenhouse on legume seedlings killed by the fungus. In the laboratory, the fungus sporulates on various types of natural media. Leaves and petioles of red clover, alfalfa, soybeans, oats, or bluegrass sterilized with either propylene oxide or heat and floated on the surface of water agar constitute a satisfactory medium. Sporulation occurs in diffused light in the laboratory, but spores are not produced when cultures are incubated in continuous darkness.

In culture the fungus frequently produced a light-colored mutant that formed neither sclerotia nor spores. In order to maintain pure cultures, it is necessary periodically to make single-spore or hyphal tip transfers.

The optimum temperature for growth in culture was 30° C. with only a trace of growth at 10° or 35° C. (Table 1). The fungus required a longer period to reach its maximum growth rate at low temperatures.

Pathogenicity.—Red clover roots are attacked by a number of fungi (1, 3, 4, 5, 6, 7). Pathogenicity tests with *Leptodiscus terrestris* in comparison with other members of this disease complex, *Fusarium oxysporum* Schlecht. emend. Snyder & Hansen, *F. moniliformae* Sheld. emend. Snyder & Hansen, *F. solani* (Mart.) App. & Wr. emend. Snyder & Hansen, *F. roseum* Lk. emend. Snyder & Hansen (*F. acuminatum* Ell. & Ev.), *Phoma trifolii* Johnson & Valleau, *Gliocladium roseum* (Lk.) Bainier, and *Sclerotium bataticola* Taub., indicate that it is one of the most pathogenic members of the group.

The pathogenicity of isolates of *Leptodiscus terrestris* was tested on red clover grown in the greenhouse in steam-sterilized, artificially infested soil. The my-

TABLE 1.—*Average rate of radial growth of Leptodiscus terrestris on potato dextrose agar at 7 different temperatures*

24-hour periods	Increase in diameter of colony in mm.[a] 5°C.	10°C.	15°C.	20°C.	25°C.	30°C.	35°C.
First	0	0	0	0	.5	8	T
Second	0	0	T	4	19	26	1
Third	0	T[b]	3	15	22	29	.5
Fourth	0	T	9	12	20	25+[c]	0
Fifth	0	T	8	11	21	—	0

[a] Average of 10 plates; 2 isolates, 5 plates for each.
[b] T = trace—less than .5 mm.
[c] Growth reached the edge of the plate.

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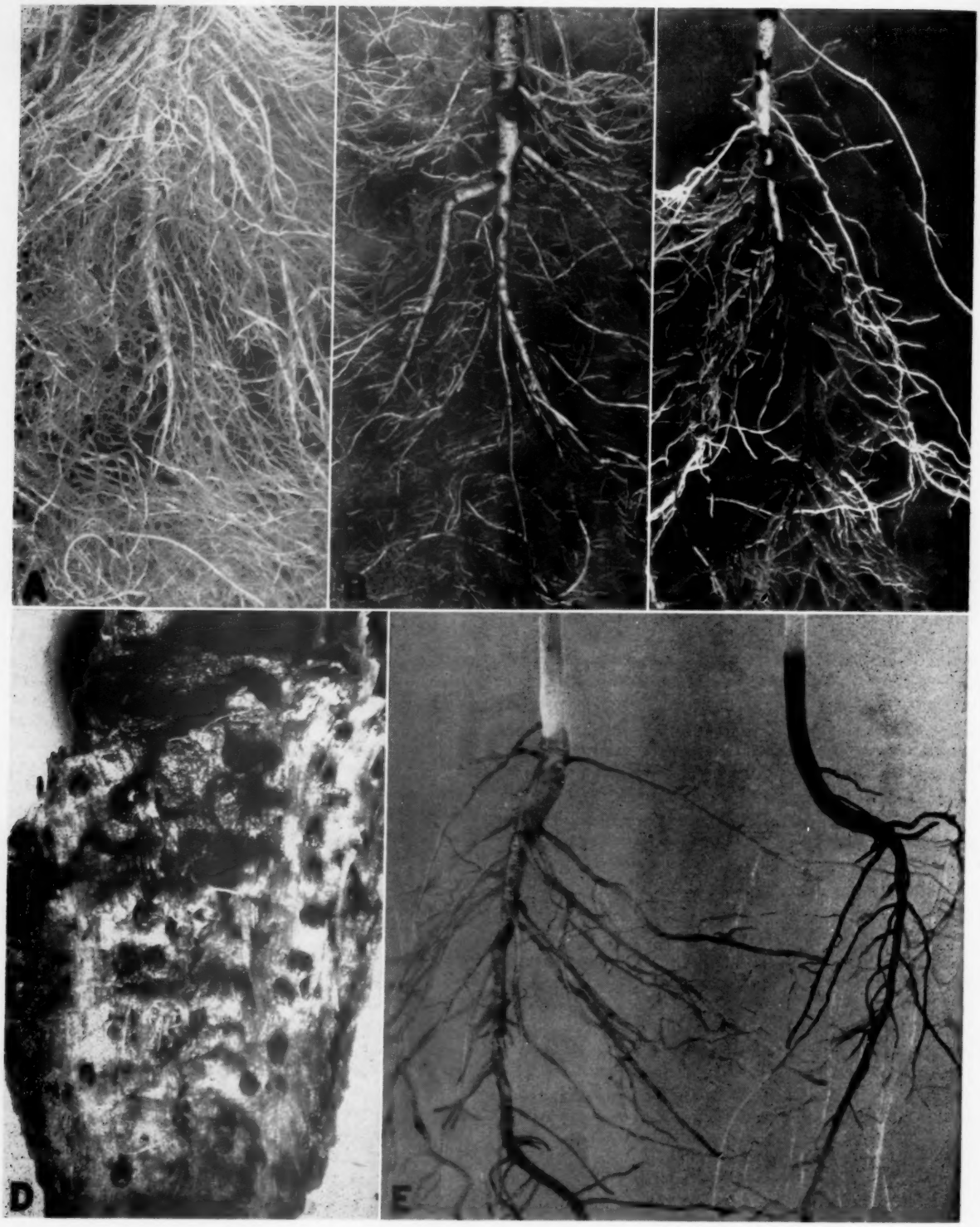

FIG. 1. A) Root of a 6-month-old red clover plant grown in steam-sterilized non-infested soil. B, C) Roots of 6-month-old red clover plants grown in steam-sterilized soil, artificially infested with *Leptodiscus terrestris*. D) Field collected, decayed red clover root containing sclerotia of *L. terrestris* ×9.7. E) Left: Root of a 25-day-old soybean grown in steam-sterilized non-infested soil. Right: Root of a 25-day-old soybean grown in steam-sterilized soil artificially infested with *L. terrestris*.

TABLE 2.—*Host range of Leptodiscus terrestris*

Plants tested		Varieties	Damping-off	Root rot	Basal stem rot	Leaf spot
Red clover	*Trifolium pratense* L.	Common; Dollard	+	+[a]	−	+
Crimson clover	*T. incarnatum* L.	Auburn; Talladega	+	+	−	——
Ladino clover	*T. repens* L.	Pilgrim	+	+	−	——
White clover	*T. repens* L.	Common	+	+	−	——
Alsike clover	*T. hybridum* L.	Common	+	+	−	——
Birdsfoot trefoil	*Lotus corniculatus* L.	N. Y. strain	+	+[a]	−	——
Lespedeza	*Lespedeza stipulacea* Maxim.	——	+	+	+	——
Sweetclover	*Melilotus alba* Desr.	Willamette; Common	+	+	−	——
Alfalfa	*Medicago sativa* L.	Commercial Colorado; Ranger	+	+[a]	−	+
Soybeans	*Glycine max* (L.) Merrill	Hawkeye	+	+[a]	+[a]	+
Garden pea	*Pisum sativum* L.	Burpeeana Early Dwarf	+	+	+	——
Green bean	*Phaseolus vulgaris* L.	Tendergreen	−	+	−	——
Lima bean	*P. limensis* Macf.	Green seeded Henderson	−	+	−	——
Oats	*Avena sativa* L.	Clinton 11	−	−	−	——
Wheat	*Triticum aestivum* L.	Pawnee	−	−	−	——
Corn	*Zea mays* L.	Ill. 1279; Ill. 1246	−	−	−	——
Cucumber	*Cucumis sativus* L.	Straight eight	−	−	−	——

[a] Found occurring naturally in the field.

celial and sclerotial growth on potato dextrose agar slants was removed with as little agar as possible, and mixed with water in a Waring Blender. This suspension was added to the soil at planting time at the rate of 1 slant per 6-in. pot. Both pre- and post-emergence damping-off occurred in infested pots. Plants that survived damping-off were left in the original pots and the roots were not disturbed in any way. The fungus penetrated the unwounded roots directly and caused a brown to black decay of the lateral root system and the taproot (Fig. 1 A, B, and C). In the field *L. terrestris* probably always occurs with other members of the disease complex; therefore, the symptoms that it causes on red clover are difficult to characterize. It can usually be recognized by a black advancing margin of decay, and by the black spherical to fusiform sclerotia that are produced in decayed tissue (Fig. 1 D).

Pathogenicity experiments also were conducted in greenhouse flats. The soil was steam sterilized and infested at the time of planting by pouring the sclerotial and mycelial growth from 1 agar slant over the seed in each 18-in. row. The results of all host range experiments are summarized in Table 2.

Alfalfa was highly susceptible to damping-off; however, it appeared much more resistant to root rot than red clover. All other small seeded legumes tested—crimson clover, Ladino clover, white clover, alsike clover, birdsfoot trefoil, lespedeza, and sweetclover—were highly susceptible to damping-off. Root rot also occurred on these crops, and although no careful evaluation of their relative susceptibility was made, in general, they appeared less susceptible than red clover. A root rot apparently caused by *Leptodiscus terrestris* was found in field-collected plants of birdsfoot trefoil (Fig. 2). It was characterized by a decay of the crown and xylem of the taproot. In some plants only a thin cylinder of functioning tissue remained. The advancing margin of decay was black and many sclerotia of *L. terrestris* were present in the decayed tissue.

In greenhouse experiments the cotyledons of soybean grown in infested soil frequently developed reddish-brown lesions and were lost prematurely. A few seedlings were killed by damping-off, and a reddish-brown to black decay developed on the roots and lower stems of remaining plants (Fig. 1, E). Sclerotia were sometimes produced in the decayed tissue. The symptoms produced on soybean roots and stems in greenhouse experiments were identical with those found in the field. The disease is difficult to distinguish from root and basal stem rot caused by *Rhizoctonia solani* Kuehn and it is sometimes necessary to isolate the causal agent for positive identification.

Green bean and lima bean appeared more resistant than soybean. A mild reddish-brown root rot developed, but no damping-off or basal stem rot occurred. Peas were sometimes killed by damping-off or root and basal stem rot. No disease symptoms were produced on wheat, oats, corn, or cucumber.

Leptodiscus terrestris was tested for its ability to produce leaf and stem infection by spraying spores on soybean, red clover, and alfalfa plants, and then placing them in a moist chamber for 48 hours. The germinating spores produced appressoria and penetration tubes pierced the epidermal cells directly. Soybean was most susceptible and small leaf spots were visible 48 hours after inoculation. These spots developed up to 3 mm. in diameter and sometimes developed along a leaf vein for as much as a centimeter in length. Lesions were produced along the full length of some petioles causing death of the leaves. The leaf spot ranged in color from reddish brown to light brown. Stem lesions were a bright reddish brown. Leaf infection developed much slower on red clover and alfalfa and the spots remained smaller. Small black lesions developed on some petioles, but they remained restricted in size and did not cause loss of the leaves. Leaf spot diseases caused by this fungus have not been

FIG. 2. Four-year-old field-collected birdsfoot trefoil plants with a root rot apparently caused by *Leptodiscus terrestris*.

found in the field. However, the spores' ability to form appressoria indicates a high degree of adaptation for this type of infection.

SUSCEPTIBILITY OF 3 VARIETIES OF RED CLOVER.—Kenland, a variety from the Kentucky Agricultural Experiment Station; Midland, a composite of midwestern strains; and Dollard, a Canadian variety originated at MacDonald College at Ste. Anne de Bellevue, Quebec, were tested for susceptibility to *Leptodiscus terrestris*. The experimental design in the following 2 experiments was a randomized block replicated 5 times with 100 seeds planted per plot. Each plot consisted of 2 18-in. rows with 50 seeds each. The soil was steam-sterilized and infested by pouring a suspension of mycelium and sclerotia from an agar slant over the seed in each row at the time of planting.

In the first experiment the flats were maintained in a warm greenhouse where the soil temperature ranged between 22° to 28° C. Emergence in the infested soil was significantly reduced, indicating the occurrence of pre-emergence damping-off (Table 3). The significant variety-treatment interaction possibly was due to a difference in seed quality rather than a varietal difference. Nearly all plants in infested soil were killed by post-emergence damping-off and all varieties were equally susceptible.

In the second experiment the soil was infested by thoroughly mixing the fungus growth from 6 agar slants into each replication (a flat 36 in. × 18 in. containing 3 in. of soil). In addition the growth from an agar slant was poured over the seed in each row at the time of planting, November 20, 1952; soil temperature was maintained between 10° and 15° C. The first results of this experiment are in Table 4. There was no reduction in emergence of seedlings in any variety, indicating that pre-emergence damping-off was controlled by soil temperatures below 15° C. On a few sunny days following emergence the greenhouse

TABLE 3.—*Percentage*[a] *emergence and post-emergence damping-off of 3 red clover varieties in infested and non-infested warm soil (22°-28° C.)*[b]

	Non-infested soil		Infested soil	
	Emergence	Post-emergence kill	Emergence	Post-emergence kill
	Per cent	Per cent	Per cent	Per cent
Dollard	82.4	0	40.4	99.1
Midland	90.4	0	62.8	100.0
Kenland	88.2	0	56.0	100.0

[a] Since the number of seeds planted per plot was 100 the percentage emergence is equal to mean emergence.

[b] For emergence, the F values for treatments, varieties, and variety treatment interaction exceed the 1 per cent level of significance.

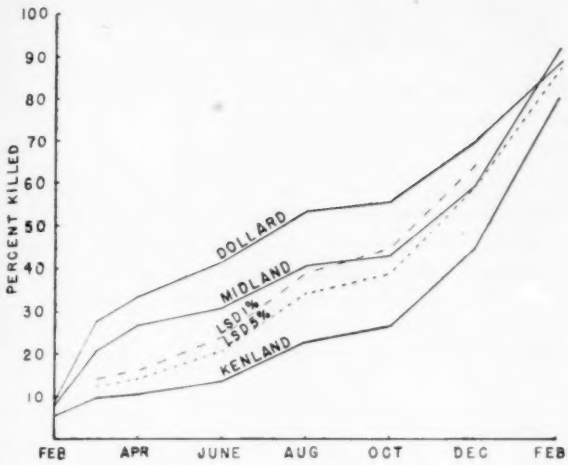

FIG. 3. Susceptibility of 3 varieties of red clover to root rot caused by *Leptodiscus terrestris*. An analysis of variance was calculated for each date. The L.S.D. lines indicate the amount a variety must differ from Kenland in order for the difference to be significant.

became warmer and several times the soil temperature became as high as 20° to 25° C. Following this exposure to warmer temperatures, a few plants damped-off, as indicated by the first reading in Fig. 3. On February 20, 1953, the experiment was moved to a warm greenhouse where the soil temperature ranged between 20° to 25° C. At this time plants began to die from root rot. By the middle of March the percentage of plants killed in the 3 varieties was significantly different (Fig. 3). Dollard was most susceptible, Midland intermediate, and Kenland most resistant. These differences were maintained through the summer and fall, but tended to become less significant when the rate of dying increased during the winter. However, at the end of a year there was still a significantly larger percentage of Kenland plants alive than in either Midland or Dollard. The decrease in rate of killing during the summer was probably due to high greenhouse temperatures which often exceeded the maximum for growth of the fungus. During the year the top growth was cut 5 times, with the greatest loss of plants always occurring immediately after cutting.

From the results of these experiments, it appears

TABLE 4.—*Percentage emergence of 3 red clover varieties in infested and non-infested cold soil (10°-15° C.)*

	Non-infested soil	Infested soil
	Emergence	Emergence
	Per cent	Per cent
Dollard	87.2	89.4
Midland	75.8	75.6
Kenland	82.2	79.2

that if the tested varieties are seeded in warm, infested soil, they are equally susceptible to damping-off. However, when they are seeded in cold, infested soil, damping-off does not occur. If the soil temperatures are raised after the plants are no longer susceptible to damping-off, root rot develops and differences in susceptibility are expressed. In Illinois, red clover is generally seeded early in the spring when the soil is cold, hence the latter conditions more nearly approximate those in the field. The resistance of the southern variety, Kenland, suggests that *Leptodiscus terrestris* may be most prevalent in the South, and that natural selection has played a part in the development of resistance.

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RESISTANCE OF HYBRID SEEDLINGS OF THE TRIFOLIATE ORANGE TO INFESTATION BY THE CITRUS NEMATODE [1]

James W. Cameron, Richard C. Baines, and Oscar F. Clarke

SUMMARY

Previous studies have shown that the common species of *Citrus* are susceptible to the citrus nematode, *Tylenchulus semipenetrans* Cobb, whereas the trifoliate orange, *Poncirus trifoliata* (Linn.) Raf., is markedly resistant. In the present study, young seedling populations of *Poncirus* from 3 sources all showed a high degree of nematode resistance. Hybrid seedling populations were obtained from crosses between *Poncirus* and 5 *Citrus* species. These populations likewise all showed marked resistance, whereas accompanying nucellar seedlings of *Citrus* were usually susceptible. Orchard trees of several older hybrids between *Poncirus* and *Citrus* have shown moderate or severe infestation by the nematode. This may reflect differences in genetic composition or a lessening of resistance in older trees.

Recent studies in California (1, 2) indicate that the citrus nematode, *Tylenchulus semipenetrans* Cobb, is a widespread pest of citrus. It seriously affects the growth of young trees planted on infested soil and apparently plays a part in the complex decline of certain older citrus orchards. Both DuCharme (3) and Baines et al. (2) found that all the common species of *Citrus* are highly susceptible to the citrus nematode. They point out, however, that the trifoliate orange, *Poncirus trifoliata* (Linn.) Raf., is markedly resistant, although not immune. The trifoliate orange is itself a useful citrus rootstock, and at least 1 of its hybrids with sweet orange shows much promise for this purpose. It seemed desirable, therefore, to investigate the heritability of nematode resistance among F_1 hybrids between *Poncirus* and a number of *Citrus* species, with the ultimate goal of obtaining new nematode-resistant rootstocks. In this paper data are presented on the resistance of young hybrid seedlings from a number of intergeneric crosses, relative to the resistance of the parent types.

MATERIALS AND METHODS.—Hybrids between *Citrus* and *Poncirus* were obtained by hand pollination of emasculated *Citrus* flowers with *Poncirus* pollen. The flowers were protected from other pollen by paper-bag covers. In order to have *Poncirus* pollen available during the flowering period of the *Citrus* spp., it was sometimes stored for short periods (4).

Seedlings were grown in a greenhouse in 1-gallon cans, 2 or more to a can. When the seedlings were 3–6 in. high, the soil in the cans was uniformly infested by pouring a suspension of nematode larvae into holes 3 in. deep, made with a probe. Six to 8 holes were used per can. With the progenies of 1950, an average of 487,000 larvae were applied to each can; with the progenies of 1951 and 1952, about 278,000 larvae were used per can. Soil temperatures were regularly held between 60°F. (night) and 85° (day), a range favorable to the activity of the nematode. Wider variations in temperature occurred only rarely.

Three to 4 months after soil inoculation the roots were washed free from soil and examined under a dissecting microscope (magnification 30 ×) for nematode infestation. The degree of infestation was classified on the basis of the average number of mature female nematodes attached per linear cm. of root, as follows: 1–5 females, slight; 6–20, moderate; and 21 or more, severe. When few or no nematodes were observed on the first roots examined, the entire root system was searched before the infestation was classified as 0 or slight.

Most *Citrus* varieties produce both apomictic (nucceller) and sexual seedlings, and it is sometimes difficult to distinguish between the 2 types. However, the trifoliate character of *Poncirus* is regularly dominant over the unifoliate condition of *Citrus* (5). In the present crosses hybrid seedlings were readily distinguished from nucellar ones, since *Citrus* was always used as the seed parent. In many cases the 2 seedling types occurred in the same can, and since most *Citrus* spp. are also polyembryonic, they sometimes grew from the same seed. Such groups provided highly comparable environmental conditions for demonstrating differential resistance to infestation.

Seedlings of the trifoliate orange and of the sour orange, *Citrus aurantium* Linn., were grown and inoculated as described above. The trifoliate orange progenies of 1950 were from open pollination; however, they were almost certainly composed of nucellar and selfed seedlings, since only self pollen was available at that time. Fruiting twigs were tagged within 2 weeks, and later-maturing fruits from possible late bloom were avoided. The sour orange seedlings were also from open pollination, but this variety regularly produces a very high proportion of nucellar seedlings, and the 2 progenies listed in Table 1 were treated as such.

RESULTS AND DISCUSSION.—The earlier data from California (2) on the degree of nematode infestation of the species used as parents in the present experiments were obtained over several years. The data were based on examination both of trees naturally infested in the field and of cuttings and seedlings infested under controlled conditions in the greenhouse.

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TABLE 1.—*Infestation of seedlings of trifoliate orange (Poncirus trifoliata) and Standard sour orange (Citrus aurantium) by the citrus nematode*[a]

Parent strain and seedling type	Number of plants	Percentage of plants showing infestation[b]		
		None or Slight	Moderate	Severe
Progenies of 1950				
Poncirus (Seedlings from open pollination; very probably nucellar or selfed)				
C.E.S. 2552 (from Florida)	77	100	0	0
C.E.S. 1717 (Pomeroy)	86	100	0	0
C.E.S. 838 (Rubidoux)	222	100	0	0
Citrus (Seedlings classed as nucellar)				
C.E.S. 628 (Standard sour orange)	62	0	10	90
Progenies of 1951				
Poncirus (Seedlings from guarded selfing; nucellar or selfed)				
C.E.S. 2552 (from Florida)	95	100	0	0
C.E.S. 1717 (Pomeroy)	366	100	0	0
Citrus (Seedlings classed as nucellar)				
C.E.S. 628 (Standard sour orange)	104	12	39	49
Totals and weighted averages				
Poncirus	846	100	0	0
Citrus	166	8	28	64
Chi square = 920				
P = <0.001				

[a] Infestation after soil inoculation in the greenhouse.
[b] Degree of infestation based on average number of mature female nematodes per cm. of fibrous root: 1-5 females, slight; 6-20, moderate; 21 or more, severe.

Nearly all the *Citrus* plants examined were moderately or severely infested under both conditions. Among the *Poncirus* plants, about half of the field trees were free of infestation or only slightly infested, whereas the remainder were usually moderately but seldom severely attacked. *Poncirus* seedlings were all free of infestation or only slightly infested. *Poncirus* is considered to be a monotypic genus, but certain strain differences, such as those in tree growth habit and in flower type, can be recognized. Differences in degree of nematode infestation observed on *Poncirus* in the field seemed to be partly correlated with strain, although there were several exceptions.

As part of the present study, seedling progenies were obtained in 1950 and 1951 from 3 sources of *Poncirus* at the University of California Citrus Experiment Station. For comparison, populations of sour orange were grown and inoculated in the same experiments. Table 1 lists the parental types and the percentages of progeny seedlings infested after soil inoculation. It may be seen that out of a total of 846 *Poncirus* seedlings none was more than slightly infested. Among 166 *Citrus* seedlings a combined average of only 8 per cent were free of infestation or only slightly infested, 28 per cent were moderately infested, and 64 per cent were severely infested. Chi square, calculated from the actual numbers of seedlings in the infestation classes of *Poncirus* as contrasted with those of *Citrus*, has a value of 920, with a probability of less than 0.001. The parent trees from which the *Poncirus* seedlings came showed differences in natural infestation: the trees of C.E.S. 2552 and C.E.S. 1717 were free of infestation, whereas the tree of C.E.S. 838 was moderately infested.

In 1951 and 1952, F_1 hybrids were obtained between *Poncirus* and 5 *Citrus* species. Table 2 lists the crosses made and the percentages of hybrid and nucellar seedlings that showed various degrees of infestation after soil inoculation. In all the crosses, hybrid seedlings were predominantly free of infestation or only slightly infested with the nematode, whereas nucellar *Citrus* seedlings commonly showed moderate or severe infestation. Eighty-six per cent of the hybrids with the Kusaie lime, for example, were free of infestation or only slightly infested, whereas only 8 per cent of the accompanying nucellar seedlings fell in this class. With the Sukega grapefruit, which is an accidental hybrid obtained at the Citrus Experiment Station by H. B. Frost, the 19 hybrids obtained were all free or nearly free of infestation. With the Clementine mandarin, 97 per cent of the 317 hybrids were likewise free or nearly free of infestation. No nucellar seedlings were obtained from the 2 last-named crosses. This is in agreement with previous evidence that both varieties are monoembryonic and produce only sexual seedlings. In the case of the Clementine, 6 rooted cuttings were tested. All 6 became moderately or severely infested. Of the total seedlings from all crosses, 95 per cent of the hybrids were free or nearly free of infestation, whereas only 18 per cent of the nucellar plants fell in this category.

With the group of progenies tested in 1952, the first soil inoculation did not result in appreciable infestation on any of the seedlings. A second inoculation was then made, but in the intervening time there had been

TABLE 2.—*Infestation of Citrus-Poncirus hybrids and accompanying nucellar Citrus seedlings by the citrus nematode*[a]

Citrus species used as seed parents[b]	Year progenies tested	Seedling type	Number of plants	Percentage of plants showing infestation[c]		
				None or slight	Moderate	Severe
C. aurantifolia, var. Kusaie lime	1952	Hybrid	36	86	8	6
		Nucellar	27	8	44	48
C. aurantifolia, var. Red lime	1952	Hybrid	53	91	9	0
		Nucellar	56	14	57	29
C. limon, var. Rough lemon	1952	Hybrid	19	95	5	0
		Nucellar	6	33	17	50
C. paradisi, var. Sukega grapefruit	1951	Hybrid	19	100	0	0
		Nucellar	0	0	0	0
C. reticulata, var. Clementine mandarin	1951	Hybrid	317	97	3	0
		Cuttings[d]	6	0	17	83
C. sinensis, var. Ruby orange	1951	Hybrid	26	100	0	0
		Nucellar	24	37	21	42
C. sinensis, var. Ruby orange	1952	Hybrid	14	93	7	0
		Nucellar	8	25	13	62
Totals and weighted averages						
		Hybrid	484	95	4	<1
		Nuclear and Cuttings	127	18	41	41

Chi square (progenies of 1951) = 207; P = <0.001
Chi square (progenies of 1952) = 128; P = <0.001

[a] Infestation after soil inoculation in the greenhouse.
[b] *Poncirus* was used as pollen parent in all cases.
[c] Degree of infestation based on average number of mature female nematodes per cm. of fibrous root: 1-5 females, slight; 6-20, moderate; 21 or more, severe.
[d] Rooted cuttings were tested, since Clementine produces no nucellar seedlings.

considerable mortality of both hybrid and nucellar seedlings, due to other causes. Only a part of the original population was thus finally available for scoring. Determinations of Chi square to compare infestation in hybrid and nucellar groups were therefore made separately for the 1951 and 1952 progenies. The Chi square values in both tests gave probability values of less than 0.001 (Table 2).

Several older representatives of earlier F_1 hybrids with *Poncirus* are available at the Citrus Experiment Station. They are in many cases hybrids produced by the early work of Webber (6) and Swingle. They occur in the form of seedling trees and rootstocks (presumably nucellar progeny of original F_1 seedlings) or trees from rooted cuttings. Contrary to the behavior of the young hybrid seedlings already described, most of these older specimens examined in the field were moderately or severely infested with the nematode. The meaning of this is not entirely clear, but it may be that trifoliate hybrids, as they grow older, do not maintain the degree of resistance which they exhibit as young seedlings. This would agree with the seemingly similar behavior of *Poncirus* itself. There may, in a few cases, be some question of the identity of these older types, since seedlings which are selected as nucellar are occasionally gametic. Where the hybrid is represented by rootstock, only, it is possible that there is some effect of the scion variety on nematode resistance. If these hybrids do gradually lose their initial resistance, they may nevertheless be of considerable value as rootstocks for the establishment of vigorous young trees during the early years of growth.

The mode of inheritance of the resistance obtained from *Poncirus* is not known, apart from the fact that it is highly dominant in F_1 hybrid seedlings. The variation in infestation observed on orchard trees of *Poncirus* might be interpreted as indicating a difference between strains in this genus, although the uniform resistance of seedlings from several sources (Table 1) does not support this. Whether or not different strains of the nematode may be involved is also unknown. Studies of this question and of the behavior of later generations of the hybrids are being undertaken.

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RELATION OF CLADOSPORIUM CUCUMERINUM TO SUSCEPTIBLE AND RESISTANT CUCUMBER TISSUE [1]

C. F. Pierson and J. C. Walker

SUMMARY

Disease development was traced in susceptible and resistant cucumber tissues inoculated with *Cladosporium cucumerinum* Ell. & Arth. National Pickling was used as the susceptible and Maine No. 2 as the resistant variety. The fruits, stems, leaves, and petioles of both varieties were inoculated by spraying with a spore suspension and were placed in a moisture chamber at 16°C. Susceptible plants were also incubated at 23° and 27°.

In the susceptible variety at 16°C. penetration occurred directly through the cuticle of fruit, stem, leaf, and petiole from well defined appressoria within 48 to 72 hours. The fungus usually progressed between epidermal cells and advanced intercellularly. Most rapid advance was in the intercellular spaces of the pith of stems and petioles and along the vascular strands of all organs. After 72 to 120 hours, depending upon the organ concerned, an intracellular habit was assumed. The invaded cells appeared to have been killed by enzymatic action prior to invasion. Subsequent collapse and lysis of cells led to the formation of gum and cavities in the parenchymatous tissues, and to the development of sunken lesions.

When inoculated susceptible plants were held at 23°C., the invading hyphae shifted slightly earlier to an intracellular habit in the stem. Moreover, the extent of cell collapse and gum formation in both stem and leaf was decidedly less than at 16°. At 27° penetration was less frequent than at 23°. The fungus was usually checked at 96 hours in the leaf with the collapse of the epidermal cells and sometimes with collapse and wall thickening of parenchyma cells. At this temperature stem lesion development ceased at 120 hours, and no gum was formed.

In the resistant variety at 16° penetration was the same as that in the susceptible. The progress of the fungus was arrested by a series of host-parasite interactions which were associated with cell wall thickening and cell necrosis. The fungus rarely became intracellular, and cell collapse was slight. Sunken lesions did not develop, and no gum was formed.

Cucumber scab was first reported from New York State in 1889 by J. C. Arthur (2), who with J. B. Ellis described the organism *Cladosporium cucumerinum* Ell. & Arth. The organism was also described from Germany in 1892 as *Cladosporium cucumeris* by Frank (8) who had not had access to Arthur's original description. Simultaneously Humphrey (10) described the disease symptoms on the foliage for the first time. The first succesful inoculation experiments were carried out in 1896 by Aderhold (1), who succeeded in transmitting the organism from fruit to fruit. Infections were obtained by Pfältzer (13), Muyzenberg (12), and Dijkstra (7) by growing seedlings in inoculated soil, and over-wintering of the organism in the soil was suggested by the first 2 investigators. Bond (6) inoculated fruits through wounds. He inoculated leaves by spraying with a spore suspension and by applying drops of spore or mycelium suspension held in contact with cotton. The spraying technique was used on seedlings and fruits by Behr (5), who also inoculated fruits, stems, and petioles through small scalpel slits or by rubbing these plant parts with small inoculum-soaked swabs. Heringa-Westerhof (9), Schultz (14), and Walker (16) employed the spraying technique exclusively.

Bond (6) reported stomatal penetration in the leaves, the mycelium becoming intercellular without haustoria and without stromatic aggregations around the vascular bundles. Later the cells were killed, and conidiophores emerged through the remains of the epidermal cells. In the fruits the hyphae traversed the cell walls readily without evidence of extensive enzymatic activity. Hyphae progressed into contact with the xylem and often invaded the spiral vessels through the unthickened portion of the wall. Schultz (14) found inter- and intracellular hyphae throughout the tissues of the stem, the most rapid spread of the fungus being by way of the intercellular spaces and the vascular tissue. He reported a browning of tissues and the formation of a periderm adjacent to the lesions in some resistant plants. Behr (5) reported direct pentration of the petiole followed by an intercellular relation of the hyphae to the tissue. On fruits direct penetration from appressoria occurred within 48 hours after inoculation along with occasional penetration of the stomata. The hyphae were intercellular and traveled along the vascular strands but never became intracellular in the vessels. Cavities were produced in infected tissues by cytolysis and were surrounded by brown cell-wall fragments and a few layers of necrotic cells. The necrotic cells were distinguished by browned walls and discolored, coagulated cytoplasm. The fungus became intracellular in the dead cells.

Muyzenberg (12), in Holland, tested 21 varieties and found 5 of them to be somewhat resistant. These were either foreign varieties or little used ones which were not commercially acceptable. Schultz and Röder (15), in Germany, surveyed 104 varieties and found certain lines of the variety Delikatess to be highly resistant. Bailey and Burgess (4) examined 22

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varieties in the United States and found the variety Longfellow to be highly resistant. Using this variety as a source of resistance, Bailey (3) developed the variety Maine No. 2, the resistance of which he reported as incompletely dominant. Walker (16) used Maine No. 2 in a genetic study and presented statistics indicating that the resistance was dominant and controlled by a single gene pair. Walker, Pierson, and Wiles (17) developed 2 scab-resistant varieties, Wisconsin SR 6 and Wisconsin SR 10, from crosses between Maine No. 2 and National Pickling and Chicago Pickling, respectively.

Cucumber scab is now known as a destructive disease in the commercial cucumber growing areas of the northern United States, southeastern Canada, and northwestern Europe when environmental conditions favor its development. Since efforts to control it with fungicides have met with indifferent success, the development and use of resistant varieties has come to be the most feasible remedy. As indicated above, there is no complete agreement among workers as to the nature of penetration and subsequent host-parasite interaction in resistant and susceptible varieties. The present investigation was concerned with this phase of the subject. The study was made with National Pickling as the susceptible and Maine No. 2 as the resistant variety. They were chosen because they have been shown (16) to differ in being homozygous for the recessive and dominant alleles, respectively, of the gene pair controlling susceptibility and resistance.

MATERIALS AND METHODS.—Seedlings of National Pickling and Maine No. 2 in the first true leaf stage, older plants in the fifth leaf stage, and both attached and detached fruits were used. A virulent isolate of *Cladosporium cucumerinum* obtained from infected fruits near New London, Wisconsin, was used throughout these studies. Stock cultures were maintained on potato-cerelose-agar slants covered with sterile mineral oil and stored in a refrigerator at 1°C. Pathogenicity was maintained at a high level by reisolating the fungus from artificially inoculated seedlings at 3-month intervals. Cultures for inoculation were grown on potato-cerelose-agar slants in 6-oz. medicine bottles. These slants were held at room temperature for 48 hours after seedling, during which time active growth was initiated. The cultures were then incubated at 16°C. for 5–9 days until heavy sporulation occurred. In the early phases of the work difficulty was experienced in harvesting the spores with distilled water, but this was circumvented by the addition of a small amount of a mild detergent, which insured thorough wetting of the spores.

A dense aqueous suspension of spores was applied immediately after harvesting by spraying the host material with an atomizer. In some of the earlier experiments the spores were applied to the host material dropwise and spread lightly over the surface with the tip of the index finger. In later experiments, however, this method was abandoned, since it was felt that the epidermis and epidermal hairs were being damaged and a distorted picture of penetration was being obtained. Following inoculation the plants were placed in moisture chambers at 16°C, unless otherwise indicated, and small pieces of host tissue were collected at 24-hr. intervals.

Tissues were killed and fixed in formalin-aceto-alcohol (4-3-93) and the air removed by aspiration for 30 minutes. Dehydration was accomplished by means of the tertiary-butyl alcohol series as outlined by Johansen (11). Gradual infiltration was obtained by placing the pieces of tissue in a 1:1 mixture of paraffin oil and tertiary-butyl alcohol on top of solidified paraffin in a shell vial, which was placed in the paraffin oven where the paraffin melted and the tissue sank to the bottom. This was followed by 4 changes of melted paraffin and finally by Fisher's tissuemat. All material was sectioned on a rotary microtome at 10μ. Conant's quadruple stain was used on all sections and Clarite was used for mounting the cover glasses.

Whole mounts were made by staining killed and fixed tissues with aqueous cotton blue, dehydrating in absolute alcohol, and clearing in carbol-turpentine. Permanent mounts were made by washing tissues thus handled in xylol and mounting in Clarite. The use of this technique facilitated the examination of randomly selected specimens from each tissue lot prior to the use of the more involved techniques and was also useful in surveying large numbers of specimens in determining the mode of penetration.

EXPERIMENTAL RESULTS.—The host-parasite relationship to be considered first will be confined to those observations made following incubation at 16°C. This temperature was selected because the disease progresses more slowly in the early stages than at higher temperatures and permits closer observation of the fungus in host tissues. The reactions of leaf, stem, and petiole tissues at 23° and 27° will be considered later.

Relation of the pathogen to fruit tissue.—In the susceptible variety the parasite penetrated directly from well-defined appressoria which developed at the ends of short germ tubes. Forty-eight hours after inoculation hyphae were found in or between epidermal cells (Fig. 1,A) and in the basal cells of glandular hairs (Fig. 1,B), which cover the entire surface of young fruits and the apical portions of somewhat older ones. In some cases at the end of 96 hours large subcuticular hyphae had subtended the length of as many as 8 epidermal cells (Fig. 1,C). Where the fungus entered the epidermal cell, it assumed an intercellular habit as soon as it had emerged from that cell into the hypodermis and continued in that relation into the cortex (Fig. 1,D). Rapid spread of the fungus through the host was facilitated by the presence of patches of phloem which frequently extended to within as few as 4 cell layers below the epidermis. The hyphae grew parallel to the sieve tubes in this tissue (Fig. 1,D) much more rapidly

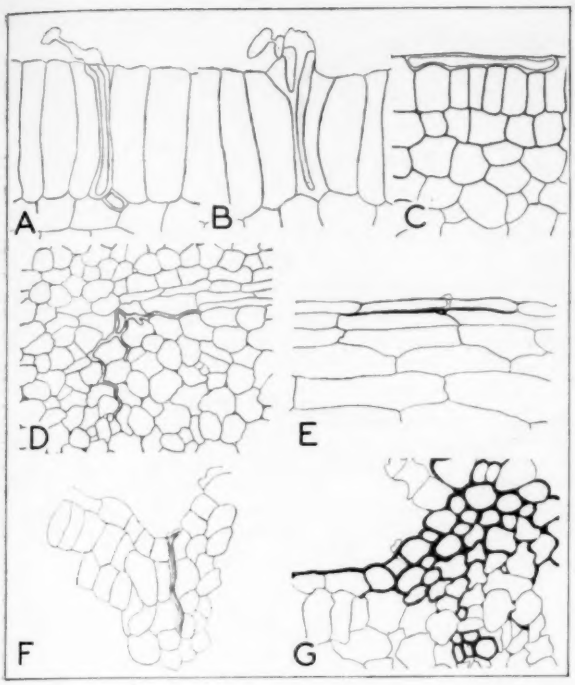

FIG. 1. A) Penetration between epidermal cells of the susceptible fruit. B) Penetration through the basal cell of a glandular hair of the susceptible fruit. C) Hypha advancing between cuticle and subcuticular wall of the subceptible fruit. D) Intercellular hypha in susceptible fruit cortex; a portion of the hypha runs parallel with and adjacent to a phloem cell. E) Penetration of epidermal cell of resistant stem; note thickening of inner epidermal cell wall. F) Penetration of susceptible leaf, showing spore, appressorium, and hypha between epidermal and palisade cells. G) Penetration of resistant leaf; note spore, appressorium, and thickening of host cell walls.

than they did when advancing between the parenchyma cells of the cortex. For the first 96 hours the habit of the fungus was entirely intercellular, and the cells adjacent to the hyphae were normal in appearance. After 96 hours the cytoplasm of cells adjacent to intercellular hyphae shrank from the cell walls and developed an abnormal staining reaction. Since a zone 4 to 5 cells in width was affected, it appeared that a substance secreted by the fungus might be responsible. Following this change in host cells many hyphae became intracellular.

While the grosser aspects of sunken lesions have been described (1, 2, 5, 6, 8), details of their development have not been given. Seventy-two hours after inoculation small, dark-green water-soaked spots appeared around the points of penetration. The spots increased in size and frequently coalesced to attain a diameter of 10 mm. or more. At the end of 120 hours these lesions became slightly sunken, and by the end of 144 hours the typical sunken lesion with the olive-green spore mass developed. After 96 hours, during which period the fungus invaded the hypodermis and the cortex, the cytoplasm of the hypodermal cells began to shrink, and the cells started to collapse. As the disease progressed, the cells collapsed completely and were compressed until the upper and lower cell walls almost lay together. At first the affected area was confined to 10 to 12 cells of epidermis around the original point of pentration, but as it extended to the cortex, the collapsed tissue was much more extensive. As the surface of the lesion became depressed, small lysigenous cavities in the affected tissues coalesced to form the large cavities which are characteristic of the disease (Fig. 2,A). In some cases the large cavity was completely filled with gum which often exuded through breaks in the epidermis and cuticle and on to the surface of the fruits. In other cases only a relatively small amount of gum was formed, of which none reached the surface of the fruit. As the cavity increased in size, the deeper cortical parenchyma underwent cytolysis while the hypodermis and the cortical cells adjacent to it remained relatively intact. The fungus grew profusely in the tissues overlying the cavity and formed a weft of inter-and intracellular mycelium. Individual hyphae grew out through the epidermal cells, became subcuticular, and gave rise to conidiophores. The conidiophores and conidia, olive-green en masse, imparted the characteristic color to the lesions.

In the resistant variety, inoculated under the same favorable conditions for infection, penetration occurred in the same manner as described for the susceptible one. Beyond this point progress was much slower and invasion much less extensive than in the susceptible variety. Before the fungus grew out of the epidermal cell, the walls of the latter became somewhat thickened, and the cytoplasm had become shrunken and slightly granular. The hypha assumed an intercellular habit in the hypodermis where there was marked granulation of the cytoplasm and thickening of the cell walls. Five days after inoculation the first 2 or 3 cell layers in the cortex were similarly affected, and the overlying hypodermal and epidermal cells appeared to have been killed completely. On the sixth day this necrosis extended to a depth of 6–8 cell layers into the cortex (Fig. 2, B), while in the susceptible host a lysigenous gummy cavity had been formed (Fig. 2, A). Although small depressed lesions were visible at this time, the advance of the fungus was completely checked, and lysigenous cavities did not develop. In isolated instances cambial activity was observed in a zone about 5 cell layers removed from the necrotic cells, but the irregularity of its appearance places its role in disease arrest in doubt. Disregarding this cambial activity entirely, the mechanism of resistance appeared to be a hypersensitive reaction of the host, which was associated with cell wall thickening and cell necrosis.

Relation of the pathogen to stem and petiole tissue.—In the susceptible variety the pathogen penetrated directly from well-defined appressoria within 72 hours after inoculation. After 96 hours the hyphae had emerged from the epidermal cell and had assumed an

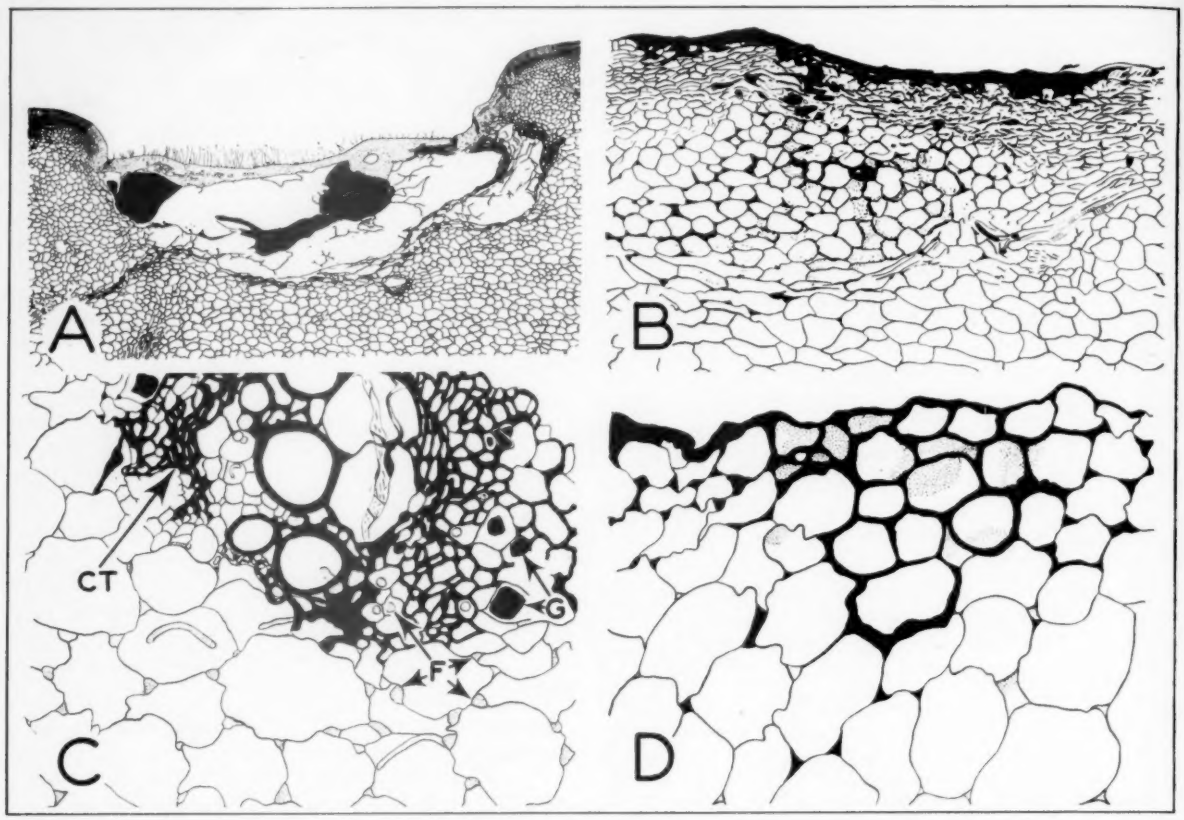

FIG. 2. A) Fruit lesion in the susceptible variety 6 days after inoculation. Note sunken epidermis, large cavity with dark margins, gum in cavity, and conidiophores arising from the upper surface of the lesion. B) Fruit lesion in the resistant variety 6 days after inoculation. Note necrosis of epidermal cells, collapse of hypodermis and cell-wall thickening in cortex. C) Susceptible stem in which the fungus (F) has advanced through the cortex, has invaded the phloem and xylem intracellularly, and has progressed intercellularly into the pith. Note collapsed tissue (CT) and gum (G). D) Resistant stem in which pronounced thickening of the walls of epidermal and cortical cells followed penetration. Stippled areas show coagulated cytoplasm. Hyphae are very sparse in such areas and do not advance beyond the lesion; none are shown in the section illustrated.

intercellular habit, extending through the cortex into the perivascular fibers and the parenchyma of the vascular cylinder. At the end of 120 hours an intracellular habit had been assumed, and the vascular bundles had been invaded. Phloem parenchyma and phloem were the most severely affected, followed in order by xylem parenchyma and xylem tubes (Fig. 2, C). Rapid spread of the fungus in the direction of the longitudinal axis of the stem occurred in the intercellular spaces of the pith parenchyma. At the end of 96 hours the cells of invaded tissues appeared to be normal, but at 120 hours the cytoplasm of many of the cells had shrunken away from the cell walls, and the cell contents had developed an abnormal staining reaction. The fungus had by this time assumed a general intracellular habit, and extensive damage had taken place. In parenchyma and collenchyma tissue of the cortex the walls of some cells were swollen, while the entire contents of others dissolved to form lysigenous cavities. The perivascular fibers and the parenchyma cells on the outer edge of the vascular cylinder were similarly invaded and dissolved to extend the cavity.

The phloem parenchyma and sieve tubes closest to the expanding cavity were among the first vascular cells to be attacked. Adjacent to the cavity the cells were either dissolved completely or collapsed to form a compact mass abutting the xylem parenchyma. At points farther from the cavity they were invaded intracellularly and remained normal in appearance except for the formation of gum within some of the cells (Fig. 2, C). Simultaneously xylem parenchyma cells were dissolved or became filled with gum. Some of the small xylem vessels were dissolved while the walls of others became swollen. Occasionally hyphae were found within the lumina of the large vessels (Fig. 2, C).

The apical portions of infected stems which were allowed to remain in the chamber for a period of 6 days became water-soaked and broke down completely. If, however, the plants were transferred to a warm, dry environment at the end of the fifth day the progress of the disease was arrested. Elliptical sunken, brown lesions appeared after 2 days in the new en-

vironment and at the end of 4 days the lesions took on a corky appearance. Unstained sections of these areas revealed a browning of cells bordering the lesion, but no cambial activity. The corky external appearance was probably due to the desiccation of collapsed tissues. Penetration and invasion of the petiole of susceptible plants were similar to that observed in stems.

In the resistant stem, penetration occurred in the same manner as described in the susceptible one (Fig. 1, E). The fungus assumed an intercellular habit, seldom went beyond the cortex, and apparently did not become intracellular. The progress of the fungus was arrested by a host reaction which brought about a thickening of the cell walls (Fig. 2, D). The exact nature of this thickening was not ascertained, but it appeared to be brought about by deposition rather than swelling.

Within 72 hours after inoculation of the resistant petiole, hyphae could be found to a depth of 3 to 4 cell layers in the cortex, and cell-wall thickening similar to that in the resistant stem was visible. In most cases the fungus was arrested completely at this stage, but occasionally it continued to grow in the cortex until at the end of 96 hours it had reached the vascular bundles. Growth of the fungus proceeded slowly in the vascular bundles, and only the parenchyma cells appeared to be affected at the end of 120 hours. No cavities were formed, and there was no invasion of the xylem tubes at 7 days after inoculation, which was 1 day longer than was required for complete collapse of susceptible petioles.

Relation of the pathogen to leaf tissue.—Penetration in the susceptible variety occurred by means of appressoria within 48 hours after inoculation (Fig. 1, F). No stomatal penetrations were observed in an exhaustive examination of many preparations. At the 48-hour period, well-defined hyphae were discernible between the cells of the epidermis and the palisade mesophyll. At 72 hours hyphae had reached the veinlets and vein endings, many of which lie just beneath the palisade mesophyll. In this vascular tissue the hyphae grew parallel to the phloem sieve tubes and the small spiral vessels. In the larger veins, where there were several layers of parnchyma and frequently 2 to 3 layers of collenchyma between the epidermis and the vascular bundles, the fungus had not yet reached the vascular tissues. At the end of 96 hours the fungus had reached the vascular bundles in all parts of the leaf, and an intracellular habit had been assumed in the parenchyma cells adjacent to them. Small gum pockets had formed in the spongy mesophyll, and larger ones 10 to 12 cells in length and 2 to 3 cells in width had formed in the softer tissues of the vascular bundles. Phloem, phloem parenchyma, and xylem parenchyma had broken down, but the fungus had not yet invaded the spiral vessels. At the end of 120 hours the disruption of phloem, phloem parenchyma and xylem parenchyma was well advanced, and the fungus had invaded the spiral vessels. Although considerable damage had been done in the vascular bundles of the leaf, it was not as extensive as that encountered in the comparable structures in fruits and stems at the end of the same period of time. Elsewhere in the blade of the leaf the fungus had become intracellular, had broken out through the epidermis, and conidiophores had started to form on the surface. In some instances the breakdown of parenchyma tissues was so severe that water-soaked spots appeared on the leaves. Fungus activity continued until the centers of the spots dropped out, or until the leaf collapsed completely.

In the resistant variety penetration was the same as described for the susceptible one. At the end of 72 hours however, there was marked thickening of host cell walls in the vicinity of the fungus (Fig. 1, G). Twenty-four hours later the thickening was more pronounced, and some of the cells had become necrotic. Five days after inoculation some of the cells had extremely thick walls, and others had collapsed slightly. At this stage further advance of the fungus was completely checked, and further incubation produced no change in symptoms.

Disease development at higher temperatures.—As pointed out earlier by Walker (16), disease symptoms appear first at 23° and 27° C., but the lesions tend to remain localized in the susceptible variety. At 16° symptoms appear somewhat more slowly, but leaf, petiole, and stem tissues collapse completely if favorable humidity is extended. Since the reaction of the susceptible plant at high temperature resembles, in some superficial aspects at least, that of resistant plants at optimum temperature, a further study of the host-parasite interaction in the susceptible host at 23° and 27° was made.

At 23° penetration of stem and leaf tissue was normal. A shift from intercellular to intracellular habit of the hyphae occurred in the leaf at 72 hours and in the stem at 96 hours. The number of hyphae and the extent of cell collapse and gum formation were decidedly less than at 16°. At 27° penetration was also normal but less frequent. In the leaf, advance of the fungus was usually checked at 96 hours with collapse of the epidermal cells. Occasionally when the fungus advanced into the parenchyma it was arrested by cell wall thickening or rapid collapse of host cells. In the stem, cavities formed in the cortex, but activity ceased at 120 hours, and no gum was formed.

DISCUSSION.—Penetration of the host was almost exclusively from appressoria which formed within 48 to 72 hours after inoculation. The isolated instances in which stomatal penetration occurred appear to be due to the fortuitous entry of the hyphae into the stomata, and this mode of penetration is seemingly of little importance in the etiology of the disease. The frequency with which Bond (6) obtained stomatal penetration in leaves may be due to the use of a nutrient solution in spore suspensions. Nutrient-infiltrated stomata could, with periodic wetting and drying of the leaves, act as foci toward which hyphae would grow. One of his figures shows a hypha in leaf tissue 8 days

after inoculation, which is 2 days after such tissues would normally be expected to decompose under optimum conditions. Behr (5) presented indirect evidence of stomatal penetration in fruits 6 days after inoculation, based on staining reactions in whole mounts, but he presented no figures showing actual stomatal penetration. Since the staining technique employed depended on the contact of hyphae with cell walls, it is possible that the ramifying hyphae within the host could have been responsible for these reactions.

Further advance may occur between or directly through the epidermal cell. It is generally agreed that the fungus next assumes an intercellular habit (5, 6, 14). The progress of the fungus is rather slow when the hyphae are advancing between the cells but is quite rapid when the vascular tissues are reached. This is particularly true in fruits and leaves. In the former, patches of phloem are scattered throughout the cortex where many of them lie 3 to 4 cell layers beneath the epidermis. In the latter, small vascular strands lie just beneath the palisade mesophyll. In the stems and petioles, lateral spread of the fungus is initially much slower than that in fruits and leaves due to several layers of collenchyma and parenchyma between the epidermis and the vascular bundles. Longitudinal spread, however, is facilitated by the intercellular spaces in the pith and by the vascular bundles, once they have been reached by the fungus. The intercellular habit is preserved for approximately 96 hours, after which an intracellular habit is also assumed. Bond (6) and Schultz (14) pointed out that the fungus became intracellular in the vessels. Behr (5), on the other hand, stated that the fungus was intercellular in the vascular bundles, and the hyphae were never found in the lumina of the trachea or tracheids. The present study showed that hyphae develop within the vessels, corroborating the findings of Bond (6) and Schultz (14).

The breakdown of susceptible host tissue is associated with drastic changes in the appearance of the cells. In the early stages a partial plasmolysis is discernible, but there is little change in the staining reaction of the cells. As the disease progresses, there is little change in the extent of the plasmolysis, but the cytoplasm becomes more dense and assumes a granular appearance. At the same time the cell walls darken, and the cells appear to have been killed. Immediately following this the cells start to collapse and become slightly compressed. This compression continues until compact masses, in which the individual cells have completely lost their identity, are formed. At the same time, the walls of adjacent uncollapsed cells are dissolved, and cavities are formed in these areas. This confirms the work of Behr (5) who stated that these cavities were formed lysigenously. The necrosis of cells and the breakdown of their walls appear to be enzymatic in nature, since cells some distance from the fungus are affected, and hyphae are found only at the margins of large cavities of advanced age. These findings are contrary to those of Behr (5) and Bond (6) who found no evidence of enzymatic activity.

The formation of gum is another feature which is associated with diseased tissue. Behr (5) showed that the gum formed was a true wound gum. Muyzenberg (12) also observed gum in diseased fruits, but felt that it could have been due to several causes. The large amounts of gum formed in diseased tissues and its absence in healthy tissue indicates strongly that it results from decomposition of cell walls or contents by enzymatic activity of the pathogen.

The outstanding feature of the resistant tissues is the series of host-parasite interactions which produces cell wall thickening and cell necrosis. This feature, which has not been reported previously in cucumber, appears to be the resistance mechanism which confines the disease to a relatively small number of host cells and prevents the formation of large lesions which are characteristic of the disease in susceptible tissues. Cell wall thickening and cell necrosis occur in all the invaded organs of the resistant host. In no case where these phenomena occur is the fungus found to be intracellular. With the exception of a few epidermal cells there is no complete collapse of cells in the resistant host. Thus it would appear that cell wall thickening prevents the collapse of affected cells and retards the formation of sunken lesions. Since it has been shown that gum formation is dependent upon the breakdown of host cells (5), the absence of gum in the resistant host may be attributed to resistant reactions which limit the breakdown of host cells. These reactions also prevent the formation of lysigenous cavities of the resistant host.

Comparison of the thickened cell walls of resistant plants with those of susceptible plants incubated at 27° C. revealed some marked differences. In the resistant host, the walls of affected cells were uniformly thick, smooth in appearance, and quite opaque. In the susceptible plants incubated at 27° the walls of affected cells were not uniformly thick, were somewhat rough and knobby in appearance, and were quite translucent. When both tissues were stained with zinc-chlor-iodide, the thickened walls of the resistant host produced the blue staining reaction of cellulose, while those of the susceptible stained a yellow-brown, indicating that the cellulose had been altered. These differences in staining reactions suggested that the thickening of cell walls of the resistant host might be owing to a deposition of material, whereas that of the susceptible cell walls might result from swelling associated with the action of a cellulolytic enzyme.

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A PARASITISM MECHANISM OF THE KENAF ANTHRACNOSE ORGANISM RELATED TO THE HYDROGEN ION CONCENTRATION IN THE TISSUES OF THE HOST

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SUMMARY

The normal vegetative aerial plant body of kenaf is composed of tissues which are for the most part decidedly acid, between pH 4.0 and 6.0. When parasitized by *Colletotrichum hibisci*, a substance or substances are secreted from the hyphae of the fungus which readily diffuse into the surrounding tissues of the host, are permeable to the cell membranes, and are strongly alkaline (pH 8.5). The pH of host tissues is rendered alkaline by these secretions, the enzyme systems are disrupted, and the tissues of the host so affected become moribund or necrotic, after which they are penetrated by the fungus. The fungus has demonstrated its ability to raise the pH of agar plates acidified with HCl from 3.0 to 8.0, and has affected the agar at some distance from the fungal colony. Availability of calcium and magnesium ions facilitate this activity.

Colletotrichum hibisci Pollacci was first described as attacking kenaf (*Hibiscus cannabinus* L.) by Hartley (3) in Java, based on Van Luijk's identification of the fungus. It was identified from field-grown kenaf in Cuba in 1952, at which time it was noted to occur more or less generally throughout the island. The organism probably had been in Cuba for some years before this, since heavy losses were experienced in 1950 (1). It is the most serious pathogen attacking kenaf in Cuba and Florida at the present time. A thorough search of the pathological literature failed to reveal any published work concerning the life cycle or growth forms of *C. hibisci*, its mode of entrance into the host, or conditions optimum to its growth and development. The present paper describes the pH relations of the fungus in cultures, and the normal pH relations of the tissues of kenaf. Data are presented which show the fungus to parasitize kenaf by shifting the pH of the tissues of the host.

It has been postulated that fungi and bacteria parasitic on higher plants may utilize the food reserve con-

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tained within a mechanically strong cell wall and a relatively impermeable plasma membrane either by mechanical penetration, or by chemical action which kills host tissue in advance, the parasite then living saprophytically on the dead tissue. Microscopic examination of microtome sections of the leaves, petioles, and stems of kenaf infected with *C. hibisci* revealed that tissue some distance from that actually inhabited by the fungus showed signs of altered metabolism, occlusion, and necrosis. These effects were greatest in tissues in immediate proximity to the fungus, and radiated out from each region of infection. Such degenerative changes in the tissues of kenaf preceded the penetration of the tissue by the fungus, and were observed to occur longitudinally as much as several cm. above and below an actual point of infection on the stem. These observations suggested that the parasitic mechanism of *C. hibisci* in kenaf is principally of a chemical nature.

While differentially staining the tissues of the host from those of the pathogen with the technique of Harrar (2), the authors observed that tissue infected by the fungus, and the tissue regions around those so infected, invariably precipitated ferric ammonium sulfate, whereas this mordanting reagent was not precipitated by normal kenaf tissue. As this salt is soluble in acid media, but precipitated when the pH rises above neutral, a pH phenomenon was suspected, and a comparison of the pH of healthy and infected tissue showed that the healthy tissues of the aerial axis were for the most part decidedly acid, but were alkaline when in contact with the fungus. A change from an acid to an alkaline pH preceded any visible damage to the tissue, was followed by visible tissue damage, and finally by penetration of the fungus. A series of experiments were initiated to study the relationships among the differences in pH of healthy and infected kenaf tissues, the pH relations of the fungus, and the ability of the fungus to parasitize the host.

pH OF THE TISSUES OF THE KENAF PLANT DURING NORMAL DEVELOPMENT.—To obtain the pH readings, fresh free-hand sections were placed in a drop of Truog triplex colorimetric pH reagent (Hellige Corp., Long Island City, N.Y.), and after a few minutes were transferred to a dry slide and examined with a hand lens. By this method the various tissues composing the organ under examination were differentially colored, and the color reaction was then translated into its pH equivalent. Confirmation of the efficacy of the method was made with other colorimetric indicators, such as Neutral Red, Brom Thymol Blue, etc. When the overall pH of a particular organ or region was desired, a free-hand section of tissue was triturated in a drop of the reagent and the reading then taken on the color of the liquid. The accurate pH range of the Truog indicator is between pH 4.0 and 8.5; it is quite possible that some of the pH values encountered extended beyond this range, but for the purposes of this study the methods were considered adequate.

OBSERVATIONS.—Five races of kenaf were included in this study, and all gave similar results. The mature, dormant kenaf seed is alkaline in all its parts, pH 8.5 or above, and after germination all parts of the seedling continue alkaline until 3 or 4 of the first true leaves have expanded. With the subsequent growth and expansion of the shoot, the epicotyl quickly becomes acid, and the pH of all leaf blades, petioles, the apical meristematic region, and cotyledons then registers 4.0. The root, including the root tip, remains alkaline to neutral, pH 8.5 to 7.0, throughout the life of the plant. In the seedling, the hypocotyl is a transitional region for pH; its tissues are acid immediately beneath the cotyledonary node, and alkaline where they merge with the root. As the plant continues development, the axial tissues in the basal part of the stem slowly tend to become neutral, and may become slightly alkaline (pH 7.5). These pH reactions are apparently effects of the metabolism of the plant, and do not appear to be directly associated with the pH of the soil in which the plant is grown.

A marked difference in pH exists between vascular and non-vascular tissues of the stem at the time the vascular cambium becomes active and secondary growth begins. Pith, cortex, and epidermis are always more acid, usually near pH 4.0 in the upper third of the axis, whereas the primary and secondary phloem and xylem become less acid, pH 5.0 to 6.5; and the vascular cambium and the most recent derivative cells lying to each side of the cambium are between pH 6.5 and 7.5.

A typical pH analysis of more mature kenaf (6 ft. vegetative plants, 14½ weeks old, with 40 elongated internodes) showed the pith, cortex, and leaves of the upper half of the stems as pH 4.0, whereas the xylem was pH 6.0 and the phloem pH 6.5. The lower half of the stems showed leaf blades of pH 4.5 to 5.0, petioles 4.0 to 5.0, pith and cortex pH 4.5 to 6.5, the xylem pH 7.0 whereas the phloem varied from pH 7.0 to 7.5. The higher pH readings were obtained in these cases from the basalmost tissues. In the roots, the phloem was most alkaline, pH 8.5, cortex pH 8.0, and xylem pH 7.5.

pH RELATIONS OF COLLETOTRICHUM HIBISCI IN CULTURES.—The work of other investigators, using other species of Colletotrichum, has shown these fungi to be capable of growth over a wide pH range. Small (5) determined that the lower limit for spore germination and growth of *C. gossypii* was pH 2.4, with pH 5.8 optimum; and *C. circinans* has been grown in a pH range from 2.6 to 8.0. Webb (7) had previously concluded that *C. gossypii* developed best in an alkaline medium, but was capable of development in acid media ranging down to pH 3.0. None of these studies, however, mentioned differences in pH between infected and noninfected tissues of the host plants.

Experiments were designed to test the growth reaction of *C. hibisci* on substrates of varying pH. A graduated pH series of corn meal agar plates was prepared in duplicate, inoculated with hyphae of the fun-

TABLE 1.—*Growth of colletotrichum hibisci on corn meal agar plates at varying levels of hydrogen ion concentration (Ca and Mg ions available in substrate).*

Initial pH of Agar	Growth Increment after 72 hours[a]	pH of Agar and Hyphae after 72 hours[b]	pH of Agar at Edge of Plate after 72 hours[c]
pH 3.0	125 mm.	pH 8.0	pH 5.0
3.5	147	8.5	6.0
4.0	145	8.5	5.2
4.5	156	8.5	6.0
5.0	144	8.5	7.0
5.5	145	8.5	7.0
6.0	140	8.5	7.0
6.5	150	8.5	7.0
7.0	152	8.5	7.0
7.5	140	8.5	7.5
8.0	139	8.5	8.0

[a] The figures given were obtained by permitting the fungus to grow out in a circle from the initial point of inoculation for 48 hours; at which time the outer limits of hyphal growth were marked with a wax pencil on the bottom of the petri dish containing the agar plate. After 72 additional hours, the increment of growth was measured along 4 radii extending outward from the previous mark to the new limits of the hyphae, and an average of these 8 measurements (4 from each of the 2 series of plates) computed and presented in the table.

[b] This pH value is for that portion of the plate covered with the fungal hyphae, in which the organism and agar are in direct contact.

[c] This pH value was taken for the agar at the extreme edge of the plate, in all cases at least 3 cm. beyond the outermost hyphal growth.

gus, and increment of growth determined after a 72-hour incubation period. The pH of the agar was adjusted for each value desired by the addition of HCl or NaOH, and a pH reading was made of each sample of agar before pouring the plates. The experiment was run in duplicate and the results, which were to all purposes identical in both sets of plates, were averaged and are presented in Table 1. These data permit several concepts of interest: 1) The hyphae and spores of *C. hibisci* are normally strongly alkaline, pH 8.5 or higher (Hyphae and spores of the fungus always gave the maximum alkaline reaction with the Truog indicator). 2) Hyphae of this fungus can initiate growth on substrates having rather wide ranges of pH, in this instance from pH 3.0 to pH 8.0. 3) The fungus secretes a substance or substances which raise the pH of acid media, and such substances are evidently powerful or are secreted in appreciable quantity, so that the substrate which is in near contact to portions of the fungus becomes as alkaline as the body of the fungus. 4) Such substances are readily soluble in agar and water and diffuse out from the fungus for appreciable distances and in appreciable quantity and materially reduce the acidity of the substrate at some distance from the fungal mass, in this instance over 3 cm. laterally. 5) No appreciable differences in rate or mode of growth among the cultures were discernible, presumably because of the fact that the fungus had quickly modified the acidity levels of the substrates until they were comparable in all cultures.

TABLE 2.—*Growth of colletotrichum hibisci on corn meal agar plates at varying levels of hydrogen ion concentration (Ca and Mg ions not available in substrate).*

Initial pH of Agar	Growth Increment after 103 hours[a]	pH of Agar and Hyphae after 103 hours[b]	pH of Agar Edge of Plate after 103 hours[c]
pH 3.0	46 mm.	pH 8.0	pH 3.0
3.5	89	8.0	3.5
4.0	102	8.0	4.5
4.5	114	8.0	5.0
5.0	119	8.5	6.5
5.5	107	8.5	5.5
6.0	129	8.5	7.0
6.5	160	8.5	7.0
7.0	185	8.5	7.5
7.5	155	8.5	7.5
8.0	135	8.5	7.5
8.5	129	8.5	8.0

[a] These figures were obtained by placing a tiny quantity of mycelium in the center of the agar plate, and permitting the fungus to grow out from this point for 103 hours, and then measuring along 4 radii at right angles to each other from the point of inoculation to the outer edge of the hyphal growth. An average of these 4 measurements for each series is given in the table.

[b] This pH value is for that portion of the plate covered with the fungal hyphae, in which the organism and agar are in direct contact.

[c] This pH value was taken for the agar at the extreme edge of the plate, in all cases at least 3 cm. beyond the outermost hyphal growth.

In this intial experiment the stock agar solution from which the pH series had been made and the plates prepared had been compounded with tap water rather than with distilled water. Since the laboratory tap water at Santiago de las Vegas contains appreciable quantities of calcium and magnesium salts, the experiment was repeated, with distilled water used throughout but with all other factors remaining much the same. These data are presented in Table 2, which shows the same general picture as summarized from Table 1. The most important difference is seen in Column 2 of the Table, which shows a definite curve for growth increment of the fungus as related to the initial and final pH of the substrate. Since such a growth curve was not secured from the agar series compounded with water which contained Ca and Mg ions, it seemed probable that the accessibility of 1 or both of these ions better enabled the fungus to regulate or alter the pH of the substrate. Sections of kenaf tissue showing damage, and sections free from infection were tested histochemically for calcium and magnesium. Significantly larger quantities of both ions, particularly calcium, were found in all tissue regions showing fungal damage. The data in Table 2 imply that growth of the fungus is optimum on nearly neutral media (pH 6.5–7.5), and that the alkaline secretions of the fungus tend to check its own growth if they accumulate in sufficient concentration. The lowering of the pH of the media outside the area covered by fungus at the upper range of the pH series is pre-

sumed to have resulted from adsorption by the agar of CO_2 released during respiratory processes of the fungus; those areas of media in contact with mycelia of the fungus in these plates remained at their initial pH or were higher.

An attempt was made to culture the fungus on a series of especially prepared buffered agars, using a similar initial pH series to that of the previous experiments, in order to confirm the optimum pH for maximum hyphal growth and development, and to study the organism's ability to overcome acidity of a buffered system. This series was buffered with the appropriate amounts of disodium phosphate and citric acid (buffers pH 3 through pH 7). The results obtained from this buffered series of cultures were poor; no measurable growth of the fungus was apparent after 103 hours in any of the plates which contained citric acid. Apparently *C. hibisci* is sensitive to, and its growth inhibited by, citric acid; this conclusion is similar to the results obtained by Tochinai (6) with *C. lini* the flax anthracnose organism, who found mycelial growth arrested by small quantities of citric and other organic acids.

Once the fungus is established in kenaf it has been observed in many cases to spread throughout the entire plant body with surprising speed. Anatomical studies by the authors have shown such rapid spread to be the result of fungal penetration of the vascular tissue and release of spores therein. When sporulation occurs in the water-conducting vessels of the xylem, the spores are of small enough diameter to be readily transported by the transpiration stream. They apparently lodge throughout the smaller ramifications of the xylem vessels and in so doing frequently establish other areas of infection in the host.

Experiments were performed to study the ability of the spores alone to germinate on and affect the pH of the substrate. A series of corn meal agar plates ranging from pH 3.0 to 8.5 were inoculated with a drop of a pure culture of spores of *C. hibisci* in water suspension. The pH of the plates had been adjusted with HCl and NaOH. Germination of the inoculum began within 4 hours, and was nearly complete on all plates after 26 hours. On substrates of pH 4.5 or lower the germinating spores had formed a mat of loose hyphae parallel to the substrate and some sporulation was evident. The acidity of the substrates ranging between pH 3.0 and 4.5 was sufficiently great to lower the pH of the fungus as the spores germinated, so that the germ tubes, hyphae, and such spores as had been produced during 26 hours after inoculation were found to be pH 4.0; the fungus had made sufficient growth, however, to retain potential menace if such germination were to occur in the kenaf plant. Germination on substrates ranging between pH 5.0 and 8.5 had resulted after 26 hours in a solid mat of hyphae on the substrate, and in addition considerable aerial growth gave the colonies the "normal" tufted appearance, with much sporulation. In this higher pH range all germ tubes, hyphae, and secondary spores were pH 8.5.

Discussion.—One of the most important known effects of hydrogen-ion concentrations in the realm of biological phenomena is the great influence of the pH of the media on the speed of reactions catalyzed by enzymes. The optimum hydrogen-ion concentration will vary for specific enzymes and may vary depending on their source, the temperature, or on other conditions. In general, however, most hydrolytic plant enzymes have maximum activity in an acid medium, whereas most oxidizing-reducing enzymes appear to function more readily in neutral to alkaline media (4). It thus seems apparent from the data at hand that *C. hibisci* parasitizes the vegetative kenaf axis by secreting a substance or substances which move through the cell membranes, and change the normally acid pH of the cell contents to the alkaline side of the scale. Such pH shifts undoubtedly exert profound effects on the enzyme systems of those tissues of the host so affected, and in consequence upset the organization of the tissue, thus bringing about metabolic changes which eventually lead to necrosis and permit entry of the fungal organism.

U. S. Operations Mission to Cuba
American Embassy
Havana, Cuba

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LONGEVITY OF UREDOSPORES OF FLAX RUST [1]

H. H. Flor [2]

SUMMARY

The longevity of uredospores of race 236 of flax rust stored in glass vials was 7 days in an unwhitewashed greenhouse (13°–43°C.), 18 days in a whitewashed greenhouse (20°–34°C.), 24 days in the laboratory (19°–25°C.), 300 days at 3°C. and −4°C., and 1295 days at −10°C. Spores survived 36 alternate coolings to −10°C. and warmings to 20–25°C. during 3½ years storage. Spore survival was similar in aerated and stoppered vials.

Studies of the genetics and physiology of pathogenicity in the rusts and of resistance in the host require the maintenance of specific uredospore cultures for several years. Often a culture must be used as a source of inoculum at frequent intervals. Consequently, suitable storage conditions must be devised to maintain the viability of the spores for the longest possible time, and yet permit the inoculum to be used intermittently. The longevity of uredospores under varying environmental conditions also determines the risks involved should exotic races be studied, and it may have a bearing on epidemiology.

Usually, rust cultures are maintained by periodic passage through the host, alternated with refrigerated storage. Hart[3] found that uredospores of *Melampsora lini* (Pers.) Lév., lost vitality most rapidly at high temperatures and at very high or very low humidities. Only a trace of germination was secured after storage for 5 weeks at 22° C., for 7 weeks at 17° C., or for 11 weeks at 7° C. Prasada[4] reported the limit of viability of uredospores stored in sealed glass tubes to be 14 weeks at 0° C., 16 weeks at 10° to 15° C., and 20 weeks at 5° to 7° C. According to Misra[5] uredospores of flax rust lost viability in a few hours at 100° to 104° F. (38° to 40° C.). Sharp and Smith[6] reported methods of lyophilization that gave excellent survival of uredospores of *Puccinia coronato* Cda. for about 500 days.

MATERIALS AND METHODS.—Uredospores of race 236 were grown on Bison, Dakota, and Koto flax in a greenhouse during the first 2 weeks in May 1950. Spores were collected in a large vial 10–14 days following inoculation and thoroughly mixed before placing approximately 1 ml. of spores into 20 ml. glass vials. The vials were tightly stoppered with corks except that 1 vial for each of the 6 conditions of storage was loosely stoppered with cotton to permit aeration. The vials were shaded from direct sunlight at all times and stored in an unwhitewashed greenhouse, a whitewashed greenhouse, on a bench in a laboratory, in a refrigerator, in the ice compartment of the refrigerator, or in a food freezer.

Spore germination was tested in tap water at room temperature. At first, germination tests were made daily but the interval between tests was increased as the study progressed. One vial was used for sampling throughout the test, although 15 vials were stored under each condition of refrigeration. To prevent moisture condensation, the test vials were kept at room temperature for at least ½ hour before sampling. When the tests indicated a decrease in the viability of the spores brought to room temperature prior to each sampling, tests were begun using another vial from which the spore sample was taken without removal from refrigeration. Tests of the spores in the refrigerated aerated vials were begun after 8 weeks of storage. Spore samples were taken from these vials without removing the vials from the refrigerated compartment.

RESULTS.—The germination tests that showed significant changes in viability are listed in Table 1.

Uredospores of flax rust are short-lived at summer temperatures. Germination of the spores held in the unwhitewashed greenhouse fell off sharply after 5 days and reached 0 in 9 days. In the whitewashed greenhouse and in the laboratory, germination declined sharply from the ninth to eleventh day. A trace of germination occurred on the eighteenth day in the whitewashed greenhouse and on the twenty-fourth day in the laboratory. There was little difference in the germination of spores stored in aerated or tightly stoppered vials.

The survival of uredospores stored at 3° C. or −4° C. was similar. There was some decrease in germination after 56 days of spores alternately raised to room temperature and refrigerated. Under continuous refrigeration, survival was somewhat better in the stoppered than in the aerated vials.

Germination of the spores stored in the food freezer at −10° C. and raised to room temperature before each sampling fell off sharply after the first day, remained relatively stable at about 5 per cent for 3 months, gradually declined to about 1 per cent after

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Field Crops Research Branch, Agricultural Research Service, U. S. Department of Agriculture, in cooperation with the North Dakota Agricultural Experiment Station.

[2] Pathologist, U. S. Department of Agriculture, located at Fargo, N. Dak.

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TABLE 1.—*Germination of uredospores of Melampsora lini after storage under different conditions*

Storage period (days)	Germination[a] of uredospores stored in: Greenhouse Unwhitewashed (13°–43°C) Aerated	Unwhitewashed (13°–43°C) Stoppered	Whitewashed (20°–34°C) Aerated	Whitewashed (20°–34°C) Stoppered	Laboratory (19°–25°C) Aerated	Laboratory (19°–25°C) Stoppered	Refrigerator (3°C) Removed[b] Stoppered	Refrigerator (3°C) Not removed[c] Aerated	Refrigerator (3°C) Not removed[c] Stoppered	Refrigerator (−4°C) Removed[b] Stoppered	Refrigerator (−4°C) Not removed[c] Aerated	Refrigerator (−4°C) Not removed[c] Stoppered	Food freezer (−10°C) Removed[b] Stoppered	Food freezer (−10°C) Not removed[c] Aerated	Food freezer (−10°C) Not removed[c] Stoppered
	%	%	%	%	%	%	%	%	%	%	%	%	%	%	%
0	30	30	30	30	30	30	30	—	—	30	—	—	30	—	—
1	30	30	40	40	40	40	50	—	—	60	—	—	60	—	—
3	30	30	30	30	40	40	70	—	—	40	—	—	5	—	—
5	10	10	20	30	50	50	70	—	—	70	—	—	5	—	20
7	1	T[d]	3	5	20	20	50	—	—	80	—	—	1	—	30
9	0	0	10	15	10	15	80	—	—	60	—	—	5	—	60
11	0	0	T	T	2	0	50	—	—	60	—	—	2	—	30
15	0	0	0	0	T	0	50	—	—	50	—	—	4	—	20
18	—	—	0	T	T	T	80	—	—	60	—	—	5	—	30
24	—	—	0	0	T	0	75	—	—	60	—	—	5	—	30
32	—	—	—	—	0	0	50	—	—	40	—	—	5	—	30
42	—	—	—	—	0	0	50	—	—	50	—	—	5	—	20
56	—	—	—	—	—	—	30	50	50	25	50	75	5	25	20
70	—	—	—	—	—	—	40	60	60	25	50	80	5	30	15
100	—	—	—	—	—	—	15	30	50	20	40	60	3	20	15
150	—	—	—	—	—	—	2	5	10	5	5	30	2	20	15
200	—	—	—	—	—	—	1	5	10	1	1	10	1	10	15
270	—	—	—	—	—	—	1	1	5	T	T	T	2	10	10
300	—	—	—	—	—	—	T	0	T	0	0	T	2	7	10
350	—	—	—	—	—	—	0	0	0	0	0	0	1	4	5
400	—	—	—	—	—	—	—	—	—	0	0	0	T	T	T
575	—	—	—	—	—	—	—	—	—	—	—	—	T	T	T
1295	—	—	—	—	—	—	—	—	—	—	—	—	T	T	T

[a] Estimated germination percentage.
[b] Vial removed from refrigerated compartment and brought to room temperature ½ hour before sampling.
[c] Spore sample taken without removing vial from refrigerated compartment.
[d] T = trace (less than 1 per cent).

1 year, and persisted as a trace for more than 3½ years. During the first year of storage the spores in vials kept frozen germinated better than those alternately frozen and thawed.

There was a decline in vigor of germination at all storage temperatures, as indicated by the length and branching of the germ tube, as the germination percentage approached a trace. This decline in vigor was less marked in the spores stored at −10° C. than at the other temperatures and the germination of occasional spores that remained viable after 3½ years' storage was quite vigorous.

A test of the 12 vials that had been kept continuously at each temperature and had not been opened was made after no germination was secured in the test vials stored at 3° and −4° C. for 350 days. Spores stored at 3° C. did not germinate, but the germination in 11 of the 12 vials stored at −4° C. ranged from a trace to 10 per cent. A trace of very weakly germinating spores were secured from 3 of the 12 vials after 385 days' storage at −4° C.

On occasions a culture has been saved by inoculating a susceptible flax when the germination test indicated that it was dead. Pots containing 8 3- to 4-in. plants of Bison flax were inoculated heavily with spores from each of the 15 vials stored for 3 years at −10° C. and having less than 0.1 per cent germination. All inoculations caused infection and from 4 to 143 pustules developed from each inoculation. In another test after 3½ years' storage at −10° C. no infection was secured from 1 vial, but the other 14 produced from 1 to 33 pustules. In this test, infection was secured with spores that had been cooled to −10° C. and warmed to 20° to 25° C. a total of 36 times in 3½ years. The 4 cultures from single pustules and 2 mass pustule cultures derived from these inoculations were pathogenically identical with the parent race 236.

DISCUSSION AND CONCLUSION.—Certain risks are involved in working with pathogenic fungi in areas where the host crop is produced commercially. Studies on disease resistance, especially those aimed at meeting changes in the pathogenicity of fungi, often require the use of races not yet established in the area. Even the use of established races involves certain risks. In southeastern North Dakota, the earliest sowing of flax is in late April or early May. The first (aecial) infections of flax rust usually are observed from late in May to the middle of June. The establishment of the uredial stage early in May would give the rust 2 or 3 additional generations to build up and might be responsible for serious crop damage. Studies herein reported indicate that work with flax rust in the greenhouse at Fargo, North Dakota, should be terminated early in April.

In the flax rust studies, 70 races are maintained to identify specific genes in the development of isogenic rust lines of flax, to screen new accessions for additional sources of rust resistance, and to determine the purity of commercial and experimental lots of seed flax. These races have been renewed twice yearly by passage through a susceptible flax. The present study has shown that they may be maintained by passage through the host once yearly, followed by storage at −10° C. This reduces both labor and possibility of contamination. Most of the inoculating is done from November through March. When a culture is renewed in November or December, the uredospores are placed in 2 vials. One of these vials is stored at −10° C. to perpetuate the race. The other is stored at 3° C., at which temperature the spores remain viable for 3 to 4 months even when frequent use subjects them to repeated alternations of refrigeration and room temperatures.

The possibility of preserving races for several years by lyophilization is being explored.

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DIPLODIA FRUIT ROT OF PEACHES[1]

W. R. Wright and M. A. Smith[2]

SUMMARY

Inspections made in Georgia during the 1951 peach season revealed the presence of *Diplodia* on 11 lots of 4 varieties of peaches in percentages ranging from 0.1 to 10.4 per cent, averaging 2.8 per cent. In July 1953, *Diplodia* was also isolated from mature peaches grown in the vicinity of Inman, South Carolina. The minimum, optimum, and maximum temperatures for growth of the peach *Diplodia* on potato-dextrose agar are 45-50°, 85°, and 102°F., respectively. Sound peaches inoculated by introducing the fungus through wounds and by spraying with spores suspended in water developed typical Diplodia rot.

The natural occurrence of Diplodia rot on Georgia peaches was first observed in 1950.[3] Further observations of the disease were made in 1951 at Fort Valley, Georgia, during the course of post-harvest treatments of peaches for the control of various transit and market diseases. Inspection of 11 lots of fruit comprising 18,317 peaches revealed *Diplodia* present in percentages ranging from 0.1 to 10.4 per cent, with an average of 2.8 per cent. Varieties examined were Pearson Hiley, Early Hiley, Southland, and Elberta. Isolations of *Diplodia* were also made during July 1953, from peaches of the Elberta variety grown in the vicinity of Inman, South Carolina. This is apparently the first report of this disease on peaches from South Carolina. This paper is the result of studies of the morphology, pathogenicity, and cultural characteristics of the causal organism.

SYMPTOMS.—In the early stages of fruit infection before mycelium is apparent the Diplodia rot lesions may be mistaken for either brown rot or Rhizopus rot. The color of the Diplodia rot lesions, however, is lighter and the skin somewhat softer to the touch than that over the brown rot lesions. The skin overlying the Diplodia lesions is more tenacious than over Rhizopus lesions which slips easily with slight pressure. When the *Diplodia* mycelium first appears on lesions it may be distinguished from *Rhizopus* mycelium by the profuse, fine, cottony growth. The mycelial growth tends to be fluffy if the relative humidity is high; under drier conditions it is more appressed.

THE PATHOGEN.—Morphological characteristics indicate that the pathogen is *Diplodia natalensis* Pole-Evans. Pycnidia tend to be gregarious both on the host and in culture, forming in stromatic masses apparently free of the substrate and gray to black in outward appearance. The pycnidia are globoid to irregular and occasionally ostiolate. Measurements made from fruiting masses developed on potato-dextrose agar disclosed pycnidia ranging from 120-600μ in diameter and averaging 307μ. Measurements of 300 pycnospores from 3 different peach isolates showed a range of 10.8-28.6μ in length and 10.4-15.6μ in width. Average size was 24.4 × 13.4μ. The spores are hyaline and continuous when young. When fully mature they are 1-septate, not constricted, dark brown, and display longitudinal striations on the exospore. The shape varies slightly between ovate and ellipsoidal. Pycnospores appear to be discharged largely before coloration or septation occurs, but an occasional pycnidium may be filled with predominately mature spores. Conidiophores were not conspicuous, the few observed being short and non-septate. A perfect stage has not been observed during the course of this study.

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[2] Assistant Pathologist and Pathologist, respectively, Agricultural Marketing Service, U. S. Department of Agriculture.

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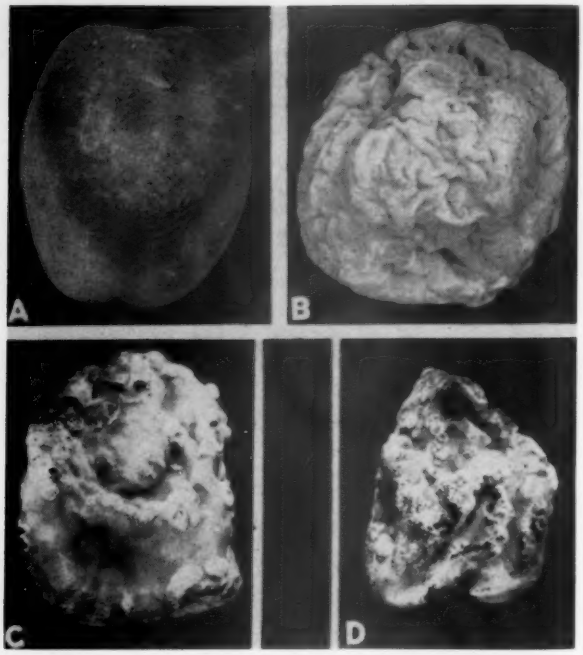

FIG. 1. Progression of Diplodia fruit rot on Elberta peaches. A) 5-day growth. B) 8-day growth. C) 13-day growth. D) 18-day growth.

The fungus is tolerant to high temperatures and produces a rapid, flocculent growth on potato-dextrose agar. A tendency toward chromogenesis characterized by a pink color of the mycelium and a diffusion of this color into the agar may be observed, particularly above 80°F.

PATHOGENICITY.—Pathogenicity of the Georgia and South Carolina peach isolates was established both in the field and in the laboratory. Inoculations of more than 100 hard-ripe Elberta peaches were made at various times, either by the insertion of inoculum from pure cultures of *Diplodia* into wounds or by spraying uninjured, brushed peaches with a water suspension of *Diplodia* spores. The infections obtained closely resembled those occurring under natural conditions.

The fungus progresses rapidly on mature fruit at high humidity and temperatures of 80-90°F. Within 4-5 days after inoculation decay is evident and a fine, white mycelial mat appears on the surface of the fruit (Fig. 1, A). At this stage infection may extend to the pit. Three or 4 days later the fruit often shows a definite beginning of mummification (Fig. 1, B). In 13 days, fruiting bodies exuding pycnospores are apparent (Fig. 1, C). Within 18 days after inoculation the peach is reduced to a shrunken mummy (Fig. 1, D). At this last stage the enveloping mycelium may still be white or taking on a smoky gray color.

Spray inoculations of hard-ripe or market-ripe peaches resulted in infection more readily with market-ripe fruit. Increased susceptibility of market-ripe fruit to infection does not seem to be due to changes in acidity of the fruit. Colorimetric tests of the reaction of the skin and flesh of immature and ripe fruits revealed little difference in their pH values. The immature and ripe skins had pH readings of 5.4 and 5.8, respectively. The pH of the pulp of the green fruit was 4.4, whereas that of the ripe fruit was 4.6. In spore germination tests there was a high percentage of germination in the juice of both green and ripe peaches.

TEMPERATURE STUDIES.—The rates of growth of *Diplodia* isolates from peach, peanut, and banana were compared in cultures at different temperatures. The isolates were grown on freshly poured plates of potato-dextrose agar adjusted to pH 6.8. Approximately 2-mm. squares of mycelium on agar were placed about 1 in. from the edges of 145-mm. petri plates and radial measurements of the growth of the fungi were made on 3 successive days.

The optimum and maximum temperatures for growth of each of the *Diplodia* isolates tested were 85° and 102°F., respectively. The minimum temperature for the growth of the peach isolate was between 45° and 50°F., for the banana isolate between 50° and 55°F., and for the peanut isolate between 55° and 60°F.

Although Diplodia rot is not yet of great importance on peaches on the market, it is potentially serious because it develops rapidly at moderate temperatures. In a shipping test of peaches under standard refrigeration, wound-inoculated fruit developed little decay during a 3½ day transit period but after the fruit was held for 3 days at room temperature, the lesions ranged from 20 to 60 mm. in diameter and averaged 37 mm. Laboratory studies and field observations showed that the fungus not only invaded peach fruit readily through wounds but that it would spread by contact to neighboring fruit. Although *Diplodia natalensis* is a common pathogen affecting many plants in the warmer climates, the source of inoculum on peach fruit has not been determined.

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VIRUS-LIKE PARTICLES IN SOLANUM TUBEROSUM[1]

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SUMMARY

The association of potato X virus with characteristic sinuous rod-like particles was investigated. Material for electron miscroscopy consisted of exudates forced from cut veins of infected plants by means of water pressure on stems or petioles. Rod-like particles were found in all parts of infected potato plants. In most potato varieties particle lengths were much greater than in 19 other species sampled.

Particles indistinguishable morphologically from those in infected plants were found in potato seedling 41956, which is immune from potato X virus. All plants of the variety examined contained the rods, in approximately similar numbers. Apparently identical rods were found in virus-free potato plants of other varieties and in most young potato seedlings. No virus could be demonstrated in these plants either by mechanical inoculation or by grafting to tomato.

Lesions produced by extracts of infected tobacco did not bear a constant relation to numbers of rod-like particles present. Lesion: particle ratios increased as extracts were diluted, but reached a figure of only 1:340 at the greatest dilution.

Rod-like particles were also found in healthy seedlings of bean, cabbage, and *Nicotiana glutinosa*.

It is believed that rod-like particles in virus-free potato plants are normal cell components. They may be involved in virus multiplication, either by conversion to virus, or as carriers of a second, infectious, component.

Purified preparations of a number of plant viruses have been found, on electron microscope examination by many investigators, to consist almost entirely of particles of a characteristic size and shape. Various lines of evidence, particularly with tobacco mosaic virus, have shown that infectivity is associated with these particles, and they have been interpreted as being virus. Wykoff (20) for example, has shown that on centrifuging, particles and infectivity are sedimented at the same rate. In tobacco mosaic virus preparations maximum infectivity is associated with rods about 280 mμ long. Newton (13) reported that ultra-sound treatment of linearly aggregated particles increased infectivity as they were broken into 280 mμ lengths. Kausche *et al.* (6) and Oster (14) showed that with longer ultra-sound treatments particles were broken into lengths shorter than 280 mμ and infectivity decreased.

The rod-like particles of potato X virus were first described by Kausche (5). He and subsequent observers (1, 4, 7, 8, 18) have described rods which were longer and more sinuous than those of tobacco mosaic virus. When the present work was started it was hoped that this characteristic shape would facilitate identification of particles in unpurified extracts.

Although the weight of evidence points to identification of virus as rod-like particles, doubts have been expressed on certain points. Johnson (4) reported, for tobacco mosaic virus, a lack of correlation in certain samples between numbers of rods and relative infectivities. In a few instances rod-like particles have been described from healthy plants (2, 10, 15).

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The present work started as an investigation of the morphology and distribution of the rod-like particles associated with potato X virus. It was soon found that apparently identical particles were present in plants believed to be virus-free. This raised a question as to the identity of rod-like particles in infected plants. The present paper concerns these particles in virus-free potatoes and their possible relation to potato X virus.

MATERIALS AND METHODS.—Plants used for infectivity tests of potato X virus preparations were *Nicotiana tabacum* L. var. Havana 38, *Gomphrena globosa* L., and *Lycopersicon esculentum* Mill. Ordinarily, production of necrotic lesions on *G. globosa* was taken as proof of the presence of potato X virus. In a number of experiments, test extracts were combined with potato Y virus and inoculated to tobacco, or combined with Holmes' masked tobacco mosaic virus and inoculated to tomato. Such virus combinations on these hosts produce distinctive and more severe symptoms than either component alone, (9) and were used to check for the possible presence of very mild strains of potato X virus.

Potato plants used came from a number of sources. Tuber-propagated plants of commercial varieties known to be carrying only potato X virus were used extensively. In addition, the X-immune seedling 41956 was obtained from New York, North Dakota, Wisconsin, and Maine. Virus-free Chippewa potato plants, and plants grown from true seed were also used.[2]

All plants were grown in 4-inch pots of composted soil at greenhouse temperatures of 25-28° C. Potato seed was sown in steamed vermiculite and the seedlings transplanted to pots of steamed soil.

Two strains of potato X virus were used; a clear ringspot strain (9), and a mild strain common to many commercial potato varieties. Virus extracts were prepared by grinding infected leaves in a mortar

[2] Thanks are due Dr. G. W. Stokes and the Wisconsin Potato Improvement Project for supplying much of the potato material.

and filtering through cheesecloth. Dilutions were made with distilled water. In many cases, inoculations were made by wiping carborundum-dusted leaves with a gauze pad saturated with inoculum. When the amount of inoculum was small, ground glass spatulas were used. All plants, except those for lesion counts, were inoculated when small and vigorously growing.

Samples for electron microscopy were usually exudates forced from cut leaf veins by water pressure on cut stems or petioles using the method described by Johnson (4). Exuded droplets were collected in capillary pipettes, and ½ of the sample was immediately placed on an electron microscope screen covered with a Formvar membrane. After about 1 minute most of the droplet was removed. The remaining liquid dried rapidly, and it is believed that this material was subject to a minimum of changes after its removal from the plant. The other half of the exudate in the capillary tube was used for inoculation. The term exudate is used to refer to liquid collected by the water pressure method, and the term extract refers to preparations from ground tissue.

All screens were kept in a desiccator over anhydrous $CaCl_2$ for about 24 hours and then shadowed with uranium. The RCA model EMU electron microscope was used, and 15-20 grid squares of each screen were viewed.

EXPERIMENTAL RESULTS.—*Rod-like particles in plants infected with potato X virus.*—In preliminary trials, various organs of naturally-infected Bliss Triumph potatoes were sampled. Characteristic particles were found in all samples, but were apparently less numerous in sprout, tuber, and rhizome exudates than in leaf, petiole, stem, root, sepal, petal, pistil, and stamen exudates. Particle size and shape were similar in all samples. The length, however, appeared to be 5–10 times the previously described (1, 9, 17) length of 500-600 mμ.

It is possible that the method of preparing samples might have affected the particle length. Bawden and Crook (1) reported that particles obtained from mill extracts were commonly less than 250 mμ long, whereas those in sap were up to 5,000 mμ long. To determine whether the water pressure method consistently yielded longer particles, more extensive trials were run on 19 other solanaceous species. These were inoculated as small seedlings with the "clear ring" strain of potato X virus, or with an extract of naturally-infected potato leaves. For comparison exudates were examined from 15 varieties of potato, all but 1 of which were known to contain potato X virus.

An approximation of the concentration of rod-like particles was obtained by counting rods in 20 random microscope fields on about 10 screens for each species. Average particle length was determined from measurements of 20–50 particles.

Rod-like particles were found in infected plants of all the following species; the average particle length in mμ is shown in parentheses: *Capsicum annuum* L. (530), *Datura stramonium* L. (595), *D. meteloides* Dunal. (493), *Lycopersicon esculentum* Mill. (517), *Nicotiana tabacum* L. (658), *N. glutinosa* L. (760), *N. rustica* L. (672), *Physalis* sp. (531), *P. floridana* Rydb. (567), *Solanum aculeatissimum* Jacq. (627), *S. aethopicum* (582), *S. gracile* Otto (587), *S. melongena* L. (551), *S. mexicana* Sesse & Moc. (572), *S. nigrum* L. var. *quincanse* (572), *S. nigrum* var. *sensulata* (587), *S. pyracanthum* Jacq. (716), *S. verbasifolium* (640), *S. villosum* Willd. (523).

Average particle length ranged from 493 mμ in *D. meteloides* exudates, to 760 mμ in N. *glutinosa* exudates. These measurements are in fairly close agreement with previously reported lengths of 500–600 mμ. In several species some particles were found which were about double the common length, and may have been end to end aggregates of 2 normal particles. The sinuous shape was characteristic of particles from all species. There was no apparent difference in lengths of particles from plants inoculated with the 2 different strains of virus. Evidently a host factor rather than strain difference or method of sample preparation is responsible for rod lengths observed in potato. All exudates produced lesions on *G. globosa.*

There were no marked differences among species in concentrations of rod-like particles. Lowest sample counts were obtained with the 2 varieties of *S. nigrum,* which averaged about 1×10^6 particles per screen. Averages for the other species ran from 2.5 to 6×10^6 particles per screen. Micrographs illustrating the appearance and range of concentration of these particles are in Figure 1.

Exudates of the following 15 potato varieties were examined, all but 1, (seedling 41956) of which were known to be infected with potato X virus. Rod-like particles were found in all of these. The average lengths in mμ are given in parentheses following the variety name: Bliss Triumph (1,500), Green Mountain (818), Seneca (2,162), Pawnee (1,576), Chenengo (1,765), Saranac (1,576), Ontario (775), Russet Rural (946), Cobbler (2,183), Warba (1,494), White Rose (1,817), Pontiac (1,629), Russet Burbank (4,290), Kennebec (1,954), USDA seedling 41956 (1,790).

In most varieties the rod-like particles were similar to those found previously in X virus-infected Bliss Triumph. Although particles 600 mμ long or less were found, the majority were several thousand millimicrons long. No significance is attached to differences in average length where maximum lengths were very long. In exudates from 2 varieties, however, Green Mountain and Ontario, average and maximum particle length were consistently less than in other varieties. Differences in particle lengths from 2 potato varieties are in Figure 2.

It is evident that rod-like particles found in most infected potato varieties are much longer than the particles in certain other species infected with potato X virus.

Rod-like Particles in USDA Seedling 41956.—The most surprising result of the survey of potato varieties was finding numerous rod-like particles in seedling

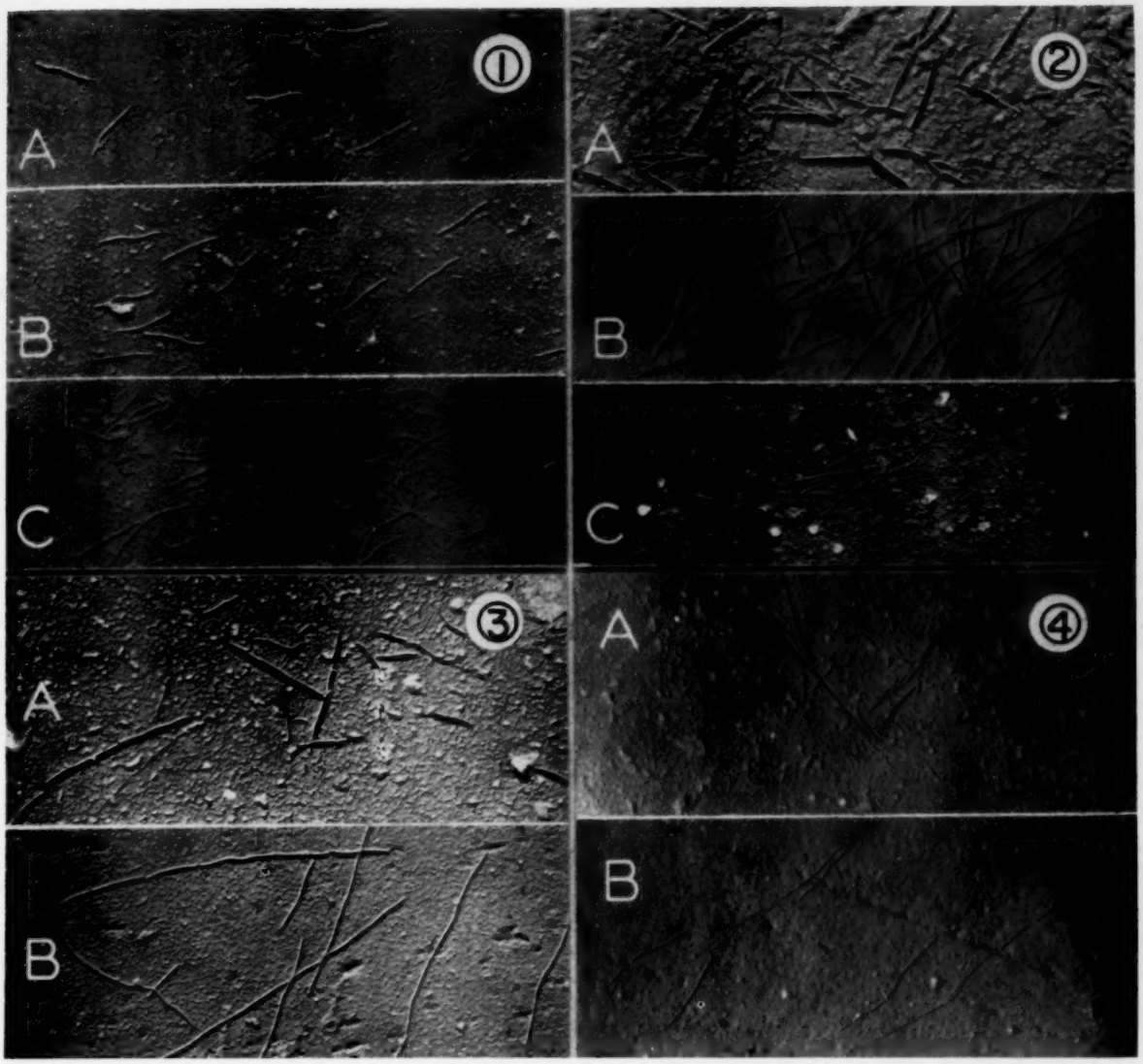

FIG. 1-4. Fig. 1. Rod-like particles in exudates of plants infected with potato virus X (clear ring strain). A) From *Solanum mexicanum* (× 18,000). B) from *S. aethopicum* (× 15,000). C) From *Lycopersicon esculentum* (× 15,000). Fig. 2. Rod-like particles in exudates of potato varieties A) Green Mountain (× 19,000), B) Bliss Triumph (× 22,000), and C) USDA seedling 41956 (× 12,000). Fig. 3. Rod-like particles in exudates of Chippewa potato. A) from plants naturally infected with potato X virus (× 18,000). B) From virus-free plants (× 19,000). Fig. 4. Rod-like particles (× 10,000) in exudates of healthy seedlings of A) cabbage (Jersey Queen) and B) bean (Pinto).

41956, shown in Figure 2, C. These were morphologically indistinguishable from particles observed in infected species. This variety is immune from potato X virus even through graft unions, as was shown in a thorough study by Clinch (3). Exudates from seedling 41956 were not infectious when tested on *Gomphrena globosa*. It seemed important to examine carefully possibilities which might account for the rods, since if this were a case of rod-like particles not representing virus, then observations on similar rods in virus-infected plants would be of doubtful value.

The exceptional length of rods observed in most potato varieties, including 41956, immediately suggested that these long rods were a second component, unrelated to virus. If this were true, however, then infected potato plants should have greater numbers of shorter rods than the virus-free 41956. This was not the case, as the average and minimum particle length found in 41956 was less than in many of the infected potato varieties. The concentration of rods, also, in 41956 was approximately equal to that in infected potato plants.

Another possibility was that the stock of 41956 tubers was carrying another virus which resembled potato X virus in morphology. It was thought that if this were true, some stocks might be found free of virus and free of rods. Accordingly, additional 41956 tubers were obtained from Iowa, New York, South Dakota, and

from 2 sources in Maine. Exudates from at least 20 plants from each tuber lot were examined. All were found to contain rod-like particles apparently identical to those previously observed. Relative numbers of particles varied from plant to plant, but average lengths and range of lengths corresponded to those of rods observed in exudates of varieties containing potato X virus.

Portions of exudates collected also were inoculated to *Gomphrena globosa* to test for infective pota o X virus. In no case did infection result. In other trials, leaf tissue from each of 30 41956 plants was combined with Holmes' masked tobacco mosaic virus and inoculated to tomatoes. These plants developed slight, if any, symptoms and were similar to tomato plants inoculated with only this strain of tobacco mosaic virus. Tomato plants inoculated with both masked tobacco mosaic virus and potato X virus showed typical necrotic streaking. Similar trials were made by combining leaf extracts of 50 41956 plants with potato Y virus and inoculating to tobacco. In no case did symptoms appear which were different from those of control plants inoculated with potato Y virus alone.

The evidence indicated that seedling 41956 was not carrying any virus which could be detected by inoculations which should reveal potato X virus. Attempts were then made to inoculate tomato seedlings with extracts of 41956 leaves. No symptoms were shown by 15 mechanically-inoculated tomatoes, and exudates did not contain rod-like particles. Exudates from tomato plants inoculated with Bliss Triumph potato leaf extracts contained abundant rods.

An attempt was made by grafting to detect a virus not mechanically transmissible. Twenty-five healthy tomato scions were grafted to stems of 41956 plants. After 21 days, when the grafts were well established, both potato stocks and tomato scions were sampled for electron microscopy by the water pressure method. Characteristic rod-like particles were found in all 25 potato stocks, but were not found in tomato scions. In 7 tomato scions there were elongated bodies over 50 mμ wide and 200 to 700 mμ long. These did not resemble the rod-like particles in 41956 potato in morphology, being irregular in outline, with blunt ends and short branches. It was concluded that rod-like particles in 41956 potato did not represent virus.

Rod-like Particles in Other Virus-free Potatoes.—In order to determine whether seedling 41956 was peculiar in possessing these particles, other virus-free potato plants were examined. Virus-free Chippewa plants were obtained from the Wisconsin Potato Improvement Project. Exudates of about 30 of these plants were examined by electron microscopy, together with 30 samples from Chippewa potatoes that had been inoculated with potato X virus. In all samples, both from virus-free and from inoculated potato plants, abundant rod-like particles were found, as illustrated in Figure 3. There were no apparent morphological differences between particles from healthy and infected potato plants.

Portions of exudates from virus-infected potato plants produced characteristic symptoms on *Gomphrena globosa* and on tobacco in combination with potato Y virus. Exudates from virus-free potato plants produced no symptoms in similar tests.

As a check on the water pressure method of obtaining exudates, these trials were repated, using juice squeezed from cut ends of petioles. Results were similar to the first trials; abundant morphologically-similar rods were found in both healthy and infected potato plants. Only juice from infected plants, however, caused infection on *Gomphrena* and tobacco.

Presence of Rod-like Particles in Potato Seedlings.—It thus appeared that rod-like particles were characteristic of all potato plants, regardless of the presence or absence of virus. To obtain further evidence of this, exudates of potato plants grown from true seed were examined. Seed from 5 potato selections was sown and the seedlings were sampled when they were 6–7 in. tall. Ten or more seedlings from each lot of seed were sampled.

All plants from 1 selection were found to contain characteristic rod-like particles. Half or more of the plants in each other lot also contained these particles. No exudate caused infection of *Gomphrena* or tobacco.

Numbers of rods from individual seedlings varied from numerous to few. It seemed possible that had more samples been taken from the other seedlings, or had they been allowed to grow to a larger size, rod-like particles might have been found.

Correlation of Infectivity with Numbers of Rod-like Particles.—The occurrence of rod-like particles in virus-free potato plants makes questionable the identity of similar rods in infected plants. Conceivably these might not be virus, or part of them might be virus and part normal cell constituents. Johnson (4) noted with tobacco mosaic virus that some preparations seemed to contain far more rods than might be expected on the basis of their relative infectivity.

An attempt was made to correlate the relative infectivities of extracts containing potato X virus with the numbers of rod-like particles present. Extracts of infected tobacco leaves were diluted 1:500, 1:2500, 1:12,500, and 1:62,500. All possible combinations of these dilutions were inoculated to sets of 7 tobacco plants, each with 3 leaves, in an incomplete block design.

Ten electron microscope screens were prepared from each dilution, using as nearly as possible the same amount of liquid for each screen. Rod-like particles in 20 microscope fields were counted at random on each screen. Knowing the approximate proportion of the total sample represented by the area of 1 field, the approximate number of particles per drop of sample exudate was calculated.

As a basis of comparing different dilutions, the average number of lesions per half-leaf was divided by the calculated number of particles per drop. The volume of liquid involved in each test was not known precisely, but should have been nearly the same for each test with each dilution. The results are only approximations, and are to be interpreted on a relative rather than an absolute basis.

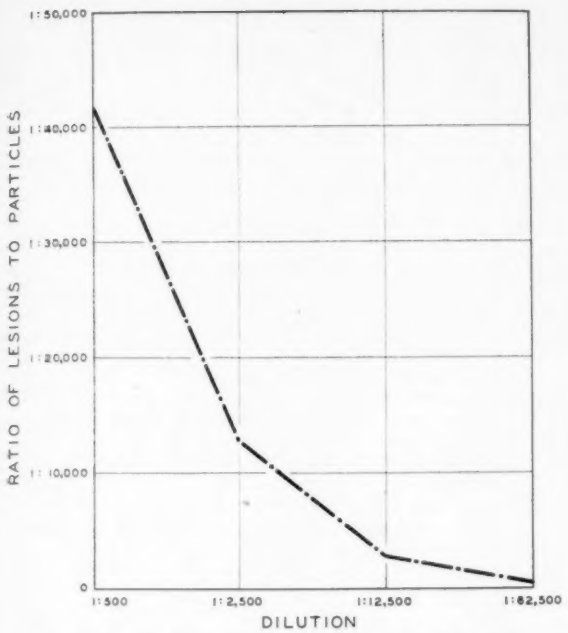

FIG. 5. Lesion/particle ratios at 5 dilutions of potato X virus extract from tobacco. The ratios were obtained by counting particles in a sample of each dilution, and inoculating the remainder to tobacco for lesion counts.

Variation in the ratios between lesions and particles at 4 dilutions is shown in Figure 5. It was apparent that for a given number of particles fewer lesions were produced at low than at high dilutions. Even at the greatest dilution, however, the average lesion:particle ratio was 1:340. Evidently a high proportion of particles were non-infectious or were unable to produce a lesion because of inadequacies of technique or host. Thus it did not seem possible to determine whether infection of potato caused a significant increase in numbers of rod-like particles present. Even assuming a relatively high efficiency of inoculation, the total amount of active virus could be represented by only a small fraction of the total number of rod-like particles present in potato plants.

Presence of Rod-like Particles in Species other than Potato.—Exudates from seedlings of a number of species were examined to determine the presence of rod-like particles. No particles were found in the following: *Nicotiana tabacum* L. (var. Havana 38), *Lycopersicon esculentum* Mill., *Petunia hybrida* Vilm., *Datura meteloides* Dunal., *Salvia farinacea* Benth., *Salvia splendens* Ker., *Veronica* sp., *Digitalis lanata* Ehrh., *Linaria maroccana* Hook., *Zinnia elegans* Jacq., *Zea mays* L., *Hippeastrum hybrida, Vigna sinensis* Endl., *Daucus carota* DC., *Cucumis sativus* L., and *Vinca minor* L.

In each of 10 *Phaseolus vulgaris* L. (Pinto) seedlings and in each of 10 cabbage (Jersey Queen) seedlings rod-like particles were found, as shown in Figure 4. Similar rods were found in 6 of 10 *Nicotiana glutinosa* L. plants, previously reported by Larson, Matthews and Walker (10).

As with potato seedlings, numbers of rod-like particles, when present, varied from plant to plant. Since only a small number of relatively young plants were examined it seems possible that a more thorough examination would reveal rods in at least some species reported as lacking them.

DISCUSSION.—In the work reported here, infectivity of potato X virus was always associated with rod-like particles. Rods were present in various organs of infected plants. They appeared in large numbers in infected species, healthy individuals of which showed no such particles. In view of evidence presented by others (11, 12, 14, 16) there seems to be little doubt that virus infectivity is associated with these characteristic rod-like particles.

The presence of morphologically-identical rods in virus-free potato plants, however, raises a number of questions as to their nature and relation to potato X virus. It is believed that the evidence sufficiently eliminates possibilities that the rods were those of another virus, or that they represent an extremely mild strain of potato X virus. Their nearly universal occurrence in potato leads to the conclusion that they are normal cell constitutents.

It may be that rod-like particles in healthy potato plants are only superficially similar to infectious particles. If this were true, then a large proportion of the rod-like particles seen in diseased potato plants represent normal constituents and not virus.

The morphological similarity, however, between the rods found in healthy potato plants and those which other evidence indicates are virus suggests a connection between the two. Rod-like particles might act as precursors (19), virus multiplication being the conversion of these rods to an infectious state. Such conversion might involve a second component, as yet undetected, which becomes infective only when combined with the rod-like particles. The apparent absence of rods in certain species might be due to their occurrence in very low numbers. Or it might be that in certain plants the rod-like bodies are not formed until after virus infection. Takahashi and Ishii (17) have described a non-infective protein in mosaic-infected tobacco which in 0.1 M ammonium acetate polymerizes into rod-like particles resembling virus. They suggest that this protein may be an intermediate stage in the production of virus, and that infectivity is acquired by combination with nucleic acid.

Further studies on rod-like particles in healthy plants are necessary before their true significance is understood. It would be particularly interesting to know whether they are nucleoprotein. A more detailed comparison of chemical composition of rods from healthy and diseased plants might provide information of fundamental value. The abundance of rod-like particles in virus-free potato plants make them a favorable source of the necessary quantities of material.

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U. S. DEPARTMENT OF AGRICULTURE,
SECTION OF TOBACCO, MEDICINAL, AND SPECIAL CROPS

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PHYTOPATHOLOGICAL NOTES

Effects of Sporotrichum sp. Link on Cowpea and 4 other Leguminous hosts.[1] William C. Schnathorst.[2] While working on a problem concerning the bacterial and fungal flora of Idaho certified beans, a species of *Sporotrichum*[3] was isolated from dissected and surface-sterilized bean stems. Due to this organism's peculiar pathogenic properties, it was felt that observations reported in this paper would be of interest.

Pathogenicity of the fungus was tested on 5 species of legumes; namely, *Glycine max* Merr., *Melilotus officinalis* Lam., *Phaseolus lunatus* var. *limenanus* Burkhart, *P. vulgaris* L. var. Black Valentine, and *Vigna sinensis* Savi var. Blackeye. The fungus was grown on potato-dextrose agar until culture plates were at least half covered. Test jars were prepared in a manner similar to that described by Halpin et al.[4] except that quart jars were employed and distilled water instead of a nutrient solution was used to moisten the sand. Each experiment consisted of 2 test jars with 1 control for each test species. Plants were grown approximately 14 days. Tests were repeated at 75° and 85°F.

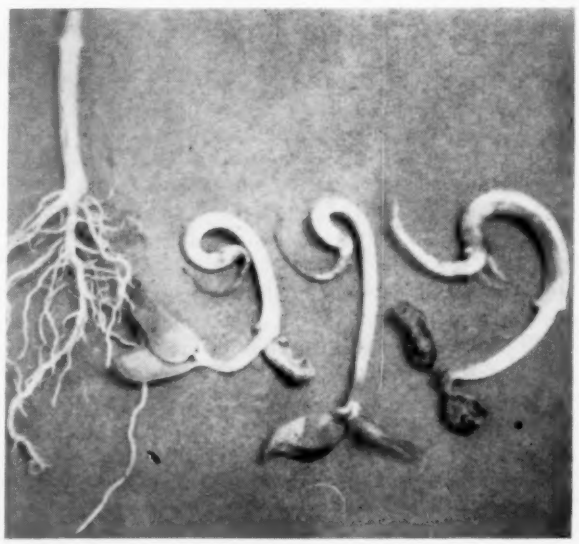

Fig. 1. Plants of *Vigna sinensis* var. Blackeye showing effects of *Sporotrichum* sp. Note inversion, absence of secondary roots, and stunting. Plant on the left is a control.

Although *Sporotrichum* sp. was pathogenic at 75° and 85°F. to all leguminous species tested, symptoms

[1] Portion of a thesis submitted in partial fulfillment of the requirements for the Master of Science degree, University of Wyoming.

Contributions from the Department of Botany and the Rocky Mountain Herbarium of the University of Wyoming No. 226.

[2] Graduate Assistant, Division of Plant Pathology, University of California, Davis, California.

[3] Thanks are due Dr. W. W. Diehl for identifying the organism.

[4] Halpin, J. E., E. W. Hanson, and J. G. Dickson. 1952. Studies on the pathogenicity of seven species of *Pythium* on red clover seedlings. Phytopathology 42: 245-249.

TABLE 1.—*Degree of stunting caused by Sporotrichum sp. at 75° and 85°F. to 5 leguminous plant species*[a]

Plant species	Temperature	Treatment	Measurements of plants in cm. Av. length	Maximum length	Minimum length
Glycine Max	85°F.[b]	infected	5.3	9.3	2.7
		control	20.4	25.0	9.2
	75°F.	infected	5.6	9.1	2.5
		control	12.7	20.0	5.0
Melilotus officinalis	85°F.	infected	3.5	8.2	1.6
		control	5.5	10.0	2.8
	75°F.	infected	2.7	8.5	0.9
		control	5.4	8.0	3.0
Phaseolus lunatus var. *limenanus*	85°F.	infected	5.4	9.2	2.5
		control	9.7	12.7	6.5
	75°F.	infected	5.5	9.0	3.5
		control	9.0	13.0	7.8
Phaseolus vulgaris var. *Black Valentine*	85°F.	infected	8.3	15.1	1.5
		control	13.8	16.3	10.4
	75°F.	infected	5.6	14.5	1.1
		control	11.4	21.0	10.5
Vigna sinensis var. Blackeye	85°F.	infected	8.3	13.2	2.5
		control	15.4	18.3	13.6
	75°F.	infected	6.5	10.7	4.0
		control	14.5	21.2	4.5

[a] Results were compiled from measurements recorded for 290 plants. Approximately 60 plants of each species were measured.

[b] Temperatures given are those recorded from internal portion of test jars.

expressed by *Vigna sinensis* var. Blackeye were most striking. The peculiar reaction of this host was a negative geotropic condition in which growth habits of the radicle and that of the epicotyl were inverted (Fig. 1). Of the plants which emerged at both temperatures, 14 per cent grew in an inverted position. Plants exhibiting this condition were usually first to emerge and did not show tendencies to arrange themselves in a normal position. Other symptoms of pathogenicity were fairly consistent at both 75° and 85°F. on all hosts tested and consisted of severe stunting, (Table 1) abnormal enlargement of the radicle, rotting at the tip of the tap root, and reduced secondary root formation.—Department of Botany, University of Wyoming, Laramie, Wyoming.

Leaf Crumple, A Virus Disease of Cotton.[1] R. C. DICKSON, M. McD. JOHNSON, AND EDWARD F. LAIRD, JR. For the past several years the leaf crumple disease of cotton has been observed in the Coachella and Imperial valleys of southeastern California. The incidence of the disease has increased throughout the summer in many seeded cotton fields. Stub (rattoon) cotton has often shown a high level of infection, sometimes reaching 100 per cent by midsummer.

Leaf crumple does not appear to stunt the plants conspicuously, nor to prevent boll set. It is not evident whether or not the disease reduces yield.

Cotton grown commercially in California is all American upland cotton, *Gossypium hirsutum* L., all of the Acala variety, and nearly all Acala 4-42. As a result, symptoms have been observed only in plants of the Acala 4-42 variety.

The primary symptom of leaf crumple (Fig. 1) is hypertrophy of the interveinal leaf tissue. There may also be some shortening of the veins in severely affected leaves. Hypertrophy may occur in only a few spots on a leaf, causing elevations 1-10 mm. in diameter, or it may involve nearly all the interveinal tissue and cause a downward curling of the leaf margins that produces an inverted cupping of the whole leaf. The hypertrophied surface is irregular because of confinement by the veinlets.

A similar hypertrophy may occur in the petals. Vein-clearing and interveinal chlorosis are sometimes observed in leaves and may be associated with this disease. No noticeable vein thickening and no enations nor foliar outgrowths occur.

Symptoms of leaf crumple are distinct from those of the leaf curl disease of cotton in Africa, which are fully described by Tarr,[2] and they appear to differ from those of other cotton virus diseases listed in his review. They are also distinct from the symptoms of crazy top or acromania of cotton found in Arizona, as

[1] Paper No. 802, University of California Citrus Experiment Station, Riverside, California.

[2] Tarr, S. A. J. 1951. Leaf curl disease of cotton. Commonwealth Mycological Institute. University Press, Oxford. 55 p.

FIG. 1. Symptoms of leaf crumple in Acala 4-42 cotton.

described by Cook[3] and later by Hope, King, and Parker.[4] Crazy top is a physiological disease caused by soil and water conditions. Cook[3] included a picture of a cotton plant with symptoms that look like those of leaf crumple, but he called the disease late-season leaf-curl (hybosis) and stated that it was caused by the feeding of the cotton aphid, *Aphis gossypii* Glover. It may be that he had leaf crumple, but failed to distinguish it from true aphid feeding injury. Leaves showing symptoms of leaf crumple may have aphids, thrips, and spider mites feeding on them.

[3] Cook, O. F. 1924. Acromania, or "crazy-top" a growth disorder of cotton. Jour. Agr. Res. 28: 803-828.

[4] Hope, C., C. J. King, and O. Parker. 1936. The effect of crazy top disorder on cotton plants and its control by irrigation management. U. S. Dept. Agr. Tech. Bul. 515: 1-43.

TABLE 1.—*Transmission of the leaf crumple virus from infected to healthy cotton plants by mixed populations of whitefly adults*

Dominant species of whitefly [a]	Number of insects	Number of Plants Tested	Number of Plants Infected	Percentage infection
Bemisia inconspicua	1924	66	11	16.7
Trialeurodes abutilonea	2250	89	33	37.1
Totals	4174	155	44	28.4

[a] These 2 species were always found in mixed populations.

Transmission of leaf crumple has been obtained only by grafting and by the transfer of adult whiteflies from diseased to healthy cotton plants. Successful grafts have always transmitted the disease, but 10 attempts at juice inoculation resulted in no infections.

Cotton plants in this area are hosts to 2 species of whitefly, *Trialeurodes abutilonea* (Haldeman) and *Bemisia inconspicua* (Quaint.). The former is ordinarily the dominant species during whitefly outbreaks, but the latter seems to be just as widespread and is sometimes the dominant species. So far a population of one without the other has not been found.

Field-collected adult whiteflies from fields showing a high incidence of leaf crumple were used for the transmission tests. Results of these tests are shown in Table 1. This virus is carried by 1 of these 2 species, and probably by both.

Preliminary attempts to infect plants other than cotton have been negative.

Efforts are being made to establish pure cultures of the 2 whitefly species, so that the vector may be exactly identified.—University of California, Citrus Experiment Station, Riverside, California.